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THE UNIVERSITY OF ALBERTA

Studies on Inhibition of Diaminopimelic Acid Metabolism and Fast Atom Bombardment Mass Spectra of Nucleosides and Nucleotides

by

Janice G. Kelland

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Doctor of Philosophy

Department of Chemistry

EDMONTON, ALBERTA

Spring 1986

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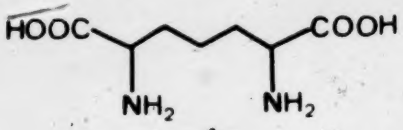
To Eric and my family

Abstract

Diaminopimelic acid (1) is an important bacterial metabolite used in the construction of cell walls and the biosynthesis of the proteinogenic amino acid lysine.



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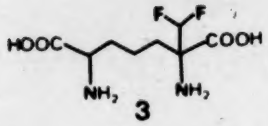
Diaminopimelic acid is not metabolised in mammals and lysine is an essential dietary component. Thus, compounds which disrupt the biosynthesis of diaminopimelic acid or its conversion to lysine *via* the pyridoxal phosphate dependent enzyme diaminopimelate decarboxylase should be effective antibiotics showing little or no mammalian toxicity. In the first part of this thesis, the synthesis of the diaminopimelic acid analogues **3**, **4** and **5** and the testing of these as potential antibacterials and inhibitors of the decarboxylase is discussed.



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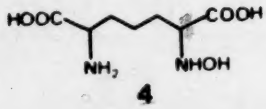

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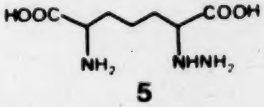

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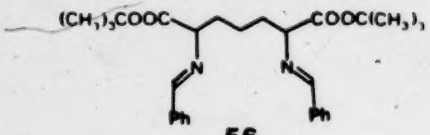
α-Difluoromethyl analogue **3** is synthesized as a mixture of isomers from diaminopimelic acid *via* anionic difluoromethylation of the dibenzylidene di-*tert*-butyl ester **56**.



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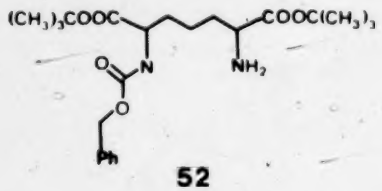
This analogue shows no antibacterial activity and is a poor inhibitor of diaminopimelate decarboxylase isolated from wheat germ or Bacillus sphaericus. It appears that the difluoromethyl group introduces too much steric bulk into the analogue to allow it to enter the active site. The N-hydroxy compound **4** is synthesized as a mixture of isomers by oxidation of the mono-N-protected diaminopimelic acid **52**.



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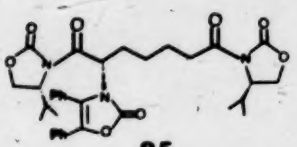
Compound **4** is a strong competitive inhibitor of diaminopimelate decarboxylase from both wheat germ and Bacillus sphaericus. It inhibits the growth of Bacillus megaterium on defined medium 75% at concentrations of 20 μg/mL but shows little or no antibacterial activity against other bacterial species grown on complex or defined media. The hydrazino analogue **5** is synthesized from α-aminopimelic acid by treatment of the anion of dioxazolidone **85** with dibenzylazodicarboxylate and deprotection of the resulting compound **93**.



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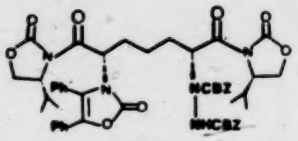

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However, epimerization occurs during this deprotection and 5 is obtained as a mixture of all isomers. This analog is a strong competitive inhibitor of diaminopimelate decarboxylase from both wheat germ and B. sphaericus and shows similar antibacterial activity to the N-hydroxy compound 4. The growth of Bacillus megaterium on defined media is inhibited 75% at concentrations of 20 μg/mL but other species are not affected.

The stereochemical course of the diaminopimelate decarboxylase reaction is also studied and both the wheat germ and B. sphaericus enzymes are shown to operate with an unusual inversion of configuration.

In the second part of the thesis, the use of fast atom bombardment mass spectrometry to study nucleosides and nucleotides is discussed. For di- and trinucleotides, cleavage occurs at the sugar-phosphate link and, in the negative ion mode, sequence information can be obtained. Protected intermediates in oligonucleotide synthesis can also be rapidly and easily characterized by this technique. Nucleosides and nucleotides containing unnatural bases behave similarly to the natural compounds and give useful fast atom bombardment mass spectra.

Acknowledgements

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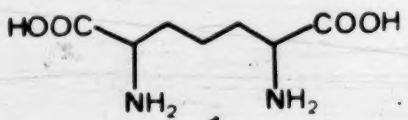
Chapter 1

Studies on Inhibition of Diaminopimelic Acid Metabolism

I. Introduction

The rational design of antibiotics requires a knowledge of the differences between bacterial and mammalian metabolism. Drugs can then be envisioned which show little mammalian toxicity while lethally disrupting the biochemical processes of the pathogen. One such difference which can be exploited is the metabolism of diaminopimelic acid (1).



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Diaminopimelic acid (2,6-diaminoheptanedioic acid, DAP) was first discovered in 1949-1950 and was isolated in 1951 from Corynebacterium diptheriae acid hydrolysates.[1] It is now known to occur in the peptidoglycan of probably all gram-negative bacteria and some gram-positive species,[2-6] and such pathogens as Klebsiella pneumoniae[7], Legionella pneumophila[8,9], Clostridium botulinum[10], Mycobacterium tuberculosis[11], Mycobacterium leprae[12], Vibrio cholerae[13] and Rickettsia quintana[14,15] contain diaminopimelic acid in their cell walls. Peptidoglycan is a net-like polymer which gives bacterial cell walls their rigidity and protects the cell from lysis due to internal osmotic pressure.[4] It consists of glycan chains of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues linked in a $\beta(1\text{-}4)$ manner, to which are attached peptide chains.

In Escherichia coli and probably all other gram-negative bacteria[2], the peptide chain is L-alanyl-γ-D--glutamyl-(L)-meso-diaminopimelyl-D-alanyl-D-alanine (Figure 1). Cross-linking occurs between the distal D center of the meso-diaminopimelyl residue of one chain and the penultimate D-alanyl residue of another chain with loss of the terminal D-alanine unit to give the rigid peptidoglycan network (Figure 2). The degree to which cross-linking occurs varies widely among species.[4]

In gram-positive bacteria, the peptide composition of the peptidoglycan can be quite different between species and is used as a taxonomic criterion.[4,16] However, in all cases known, the stereochemistry of the amino acids in the

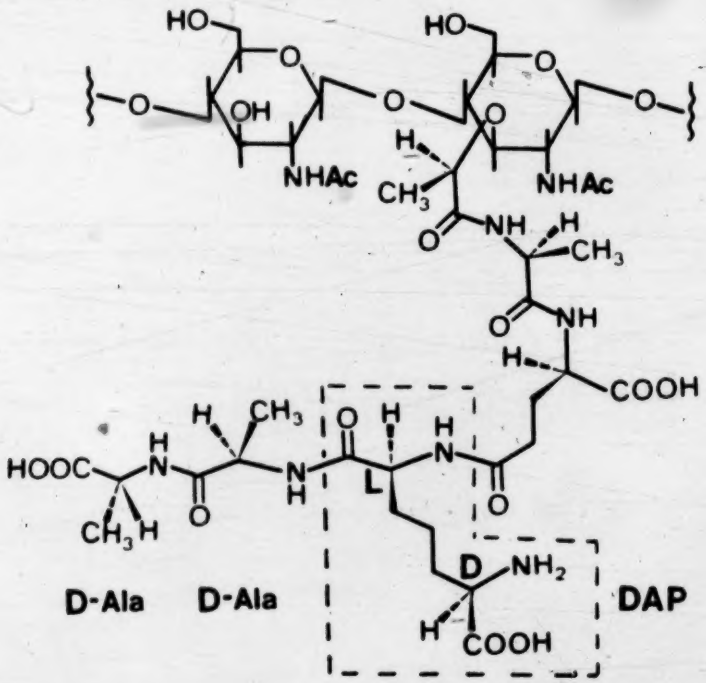


Figure 1. Peptidoglycan monomer of E. coli.

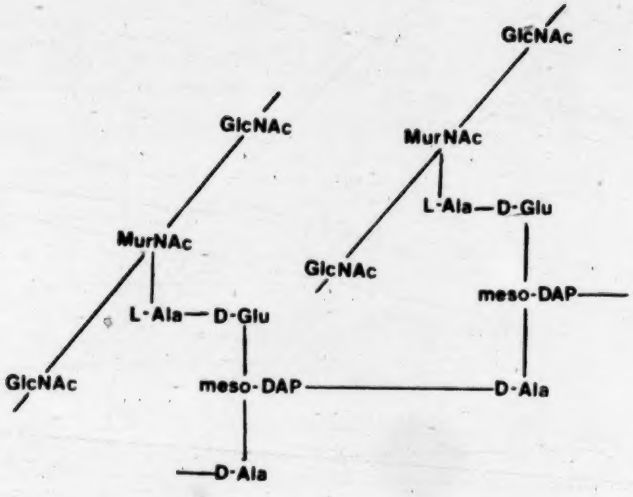


Figure 2. Crosslinking in E. coli peptidoglycan.

tetrapeptide alternates L-D-L-D.[2,4] Short oligopeptides often bridge two monomers. L-Lysine is frequently the cross-linking amino acid[5] although other diamino acids, such as meso- and LL-diaminopimelic acid[17-20] and 3-hydroxy-diaminopimelic acid[21,22] are observed as well. DD-Diaminopimelic acid has been found in Bacillus megaterium cell walls.[20,23-25]

Diaminopimelic acid is also an intermediate in lysine biosynthesis in bacteria[26-35] and plants.[36-46] The biosynthetic pathway in E. coli[26] is shown in Figure 3. In many, if not all of the Bacillus species, intermediates are acetylated rather than succinylated.[31-33] Recently, a

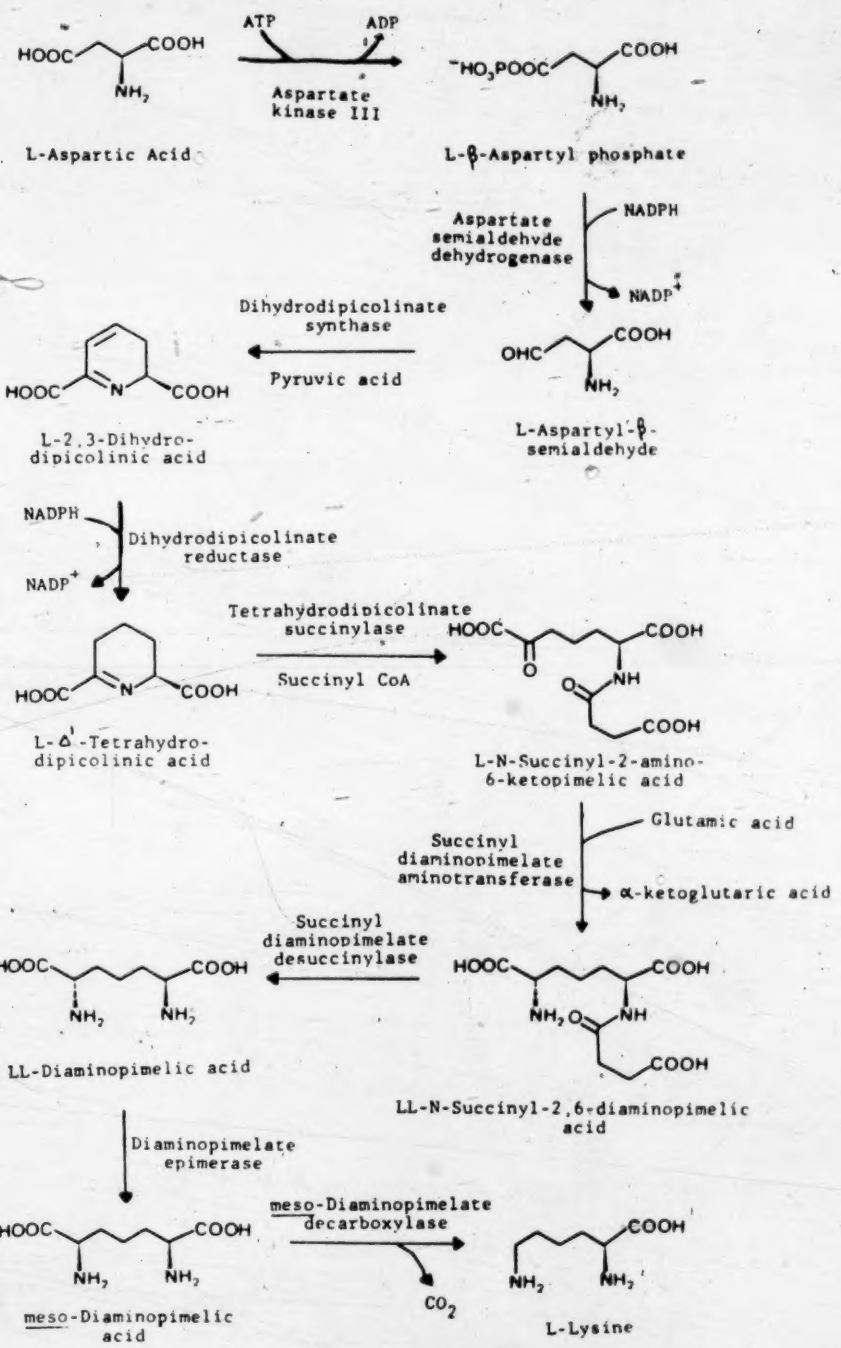


Figure 3. Lysine biosynthesis in E. coli.

modification of this pathway has been observed in Bacillus

sphaericus.[47] In this bacterium, L-tetrahydrodipicolinic acid is converted directly to meso-diaminopimelic acid by means of the enzyme meso-diaminopimelate-D-dehydrogenase.[48-51] This enzyme has also been found in other bacterial species[48] as well as in soybean embryos[43], and it is thought that Corynebacterium glutamicum may use both pathways in its lysine biosynthesis.[27,52] The B. sphaericus enzyme is dimeric and highly specific for meso-diaminopimelic acid although the DD and LL isomers are inhibitors.[50] Thiol groups are present in the active site, although these are not essential for catalysis.[51]

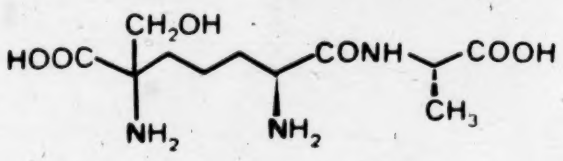
Diaminopimelate epimerase (EC 5.1.1.7) catalyses the interconversion of the meso and LL isomers of diaminopimelic acid although the DD isomer is not a substrate.[53] The enzyme has been partially purified from Bacillus megaterium[54] and maize[40] and has been purified and characterized from E. coli.[55] The enzyme does not use pyridoxal phosphate as a cofactor, unlike many other amino acid racemases[56], but it does contain an active site thiol group which is necessary for activity and may catalyse epimerization and hydrogen exchange with the medium.[55]

Diaminopimelate decarboxylase (EC 4.1.1.20) catalyses the decarboxylation of meso-diaminopimelic acid at the D-center to give L-lysine[57] and has been isolated and purified from several bacterial[28,34, 58-60] and plant[36,37,39,61-63] sources. It is the only pyridoxal phosphate dependent amino acid α-decarboxylase known to act

on the D-center of an amino acid and to operate with inversion of configuration.[64,65] It is specific for the meso isomer of diaminopimelic acid and although the DD and LL isomers are neither substrates nor inhibitors[57], lysine is a competitive inhibitor. Pyridoxal phosphate and a free active site thiol group are required for activity.[57] The gene encoding this enzyme in E. coli has been sequenced[66-68] and the corresponding amino acid sequence has thus been inferred.[26]

Diaminopimelic acid is not metabolised in mammals, who must ingest lysine in the diet, although recently, the E. coli gene for diaminopimelate decarboxylase has been successfully incorporated into mammalian cells in culture.[69] Diaminopimelic acid and small peptides containing it are rapidly excreted from mammals[70,71] and this amino acid is used as a marker of bacterial nitrogen in sheep and cattle rumens.[72-76] Ruminants can live on lysine-deficient diets, however, as ciliated protozoa in the rumen can make lysine from bacterial diaminopimelic acid.[77-79] As mammals apparently do not have diaminopimelic acid-metabolising enzymes, inhibitors of these enzymes should show little or no toxicity. However, small murein-like peptides containing diaminopimelic acid have been implicated as promoters of slow-wave sleep[71,80] and immunoadjuvants[70,81-89], and diaminopimelic acid itself is an antagonist of neuroexcitatory amino acids in rat brain[90,91] and frog spinal cord.[92]

Analogues of diaminopimelic acid which inhibit these enzymes should show antibacterial activity. In fact, the naturally occurring dipeptide antibiotic 2 contains the α-hydroxymethyl analogue of diaminopimelic acid.[93]



2

Many antibiotics such as penicillins[94] interfere with bacterial cell wall synthesis and in E. coli, diaminopimelic acid deprivation causes cell lysis.[95] Inhibitors of the epimerase or dehydrogenase should have this effect. Inhibitors of the decarboxylase will cause lysine deprivation which should be lethal, as lysine is involved in protein synthesis as well as in peptidoglycan formation in many species.[5] Active transport of exogenous lysine[96-101] and diaminopimelate[102] might bypass such blocks, but this transport might be inhibited by methylated amino acids.[103]

The catalytic mechanism of pyridoxal phosphate dependent enzymes has been extensively studied[56,104-106] and is illustrated in Figure 4 for the case of decarboxylases. The cofactor occurs as an imine adduct with the ε-amino group of an active site lysine residue[56] and transimination occurs with the α-amino group of the amino acid substrate.[107] The protonated pyridine ring now acts as an electron sink and cleavage of one of the three bonds to the

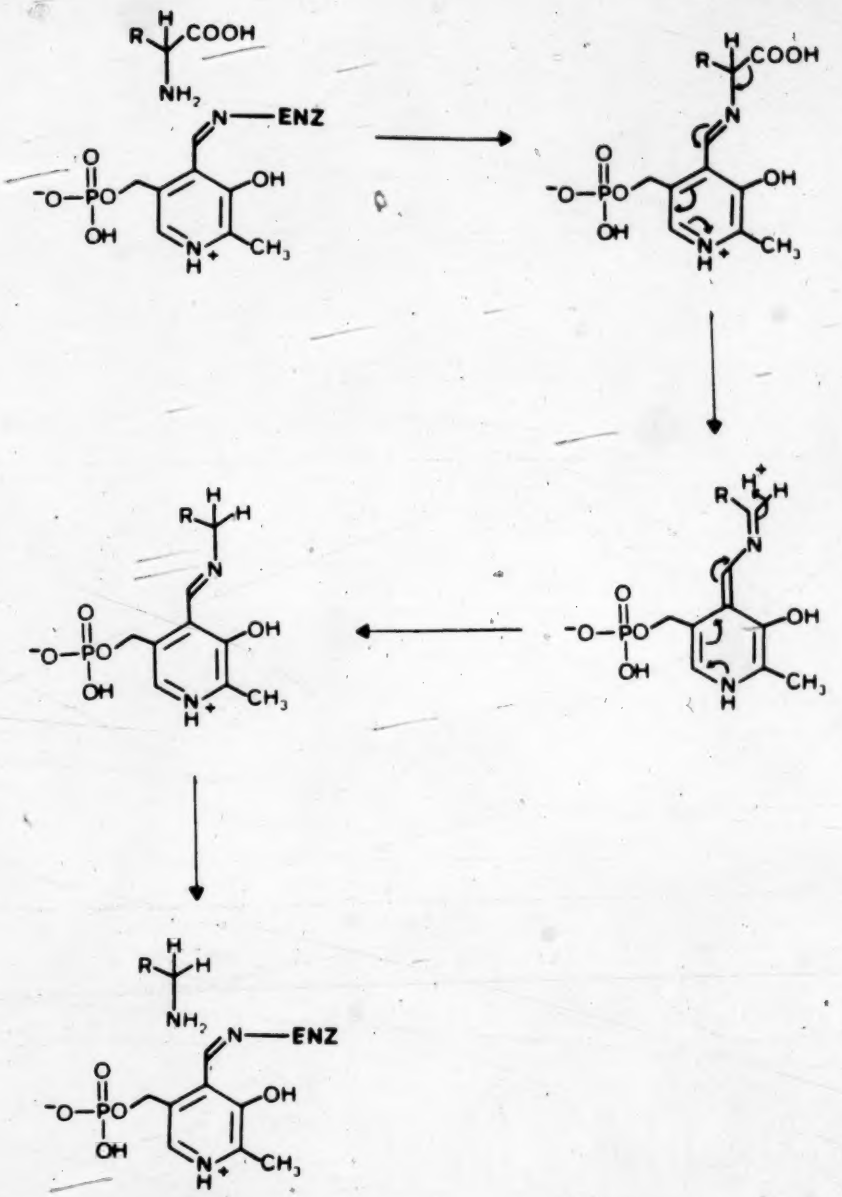
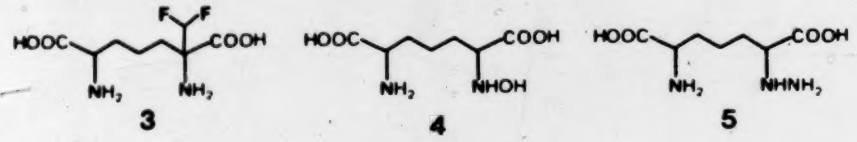


Figure 4. Mechanism of catalysis of pyridoxal phosphate dependent decarboxylases.

amino acid α-carbon can occur to leave a carbanionic intermediate. In the case of decarboxylases (Figure 4), CO_2 is lost, while for epimerases and transaminases, α-hydrogen loss takes place. The intermediate then undergoes

reprotonation and transimination or hydrolysis to release the product from the enzyme. Based on this, our synthetic targets as inhibitors of the decarboxylase are the α-difluoromethyl (3), N-hydroxy (4) and N-amino (5) analogues of diaminopimelic acid.



Several fluorinated amino acid analogues are known to be enzyme-activated irreversible inhibitors or "suicide substrates"[108-114] of pyridoxal phosphate dependent enzymes (Table 1). The mechanism by which these compounds inhibit these enzymes has been proposed[113] to be that shown in Figure 5A. Once decarboxylation occurs, the anionic intermediate can eliminate fluoride. The resulting olefin should be quite electrophilic and susceptible to attack by a nucleophilic group in the enzyme active site. This leads to covalent binding of the substrate analogue to the enzyme, blocking the approach of the true substrate. An alternative mechanism[110] could involve transimination of the unsaturated amine-pyridoxal phosphate adduct followed by attack of the nucleophilic enamine product on the electrophilic center of the cofactor. The resulting inhibitor-pyridoxal phosphate complex may be tightly bound to the enzyme, giving irreversible inhibition (Figure 5B).

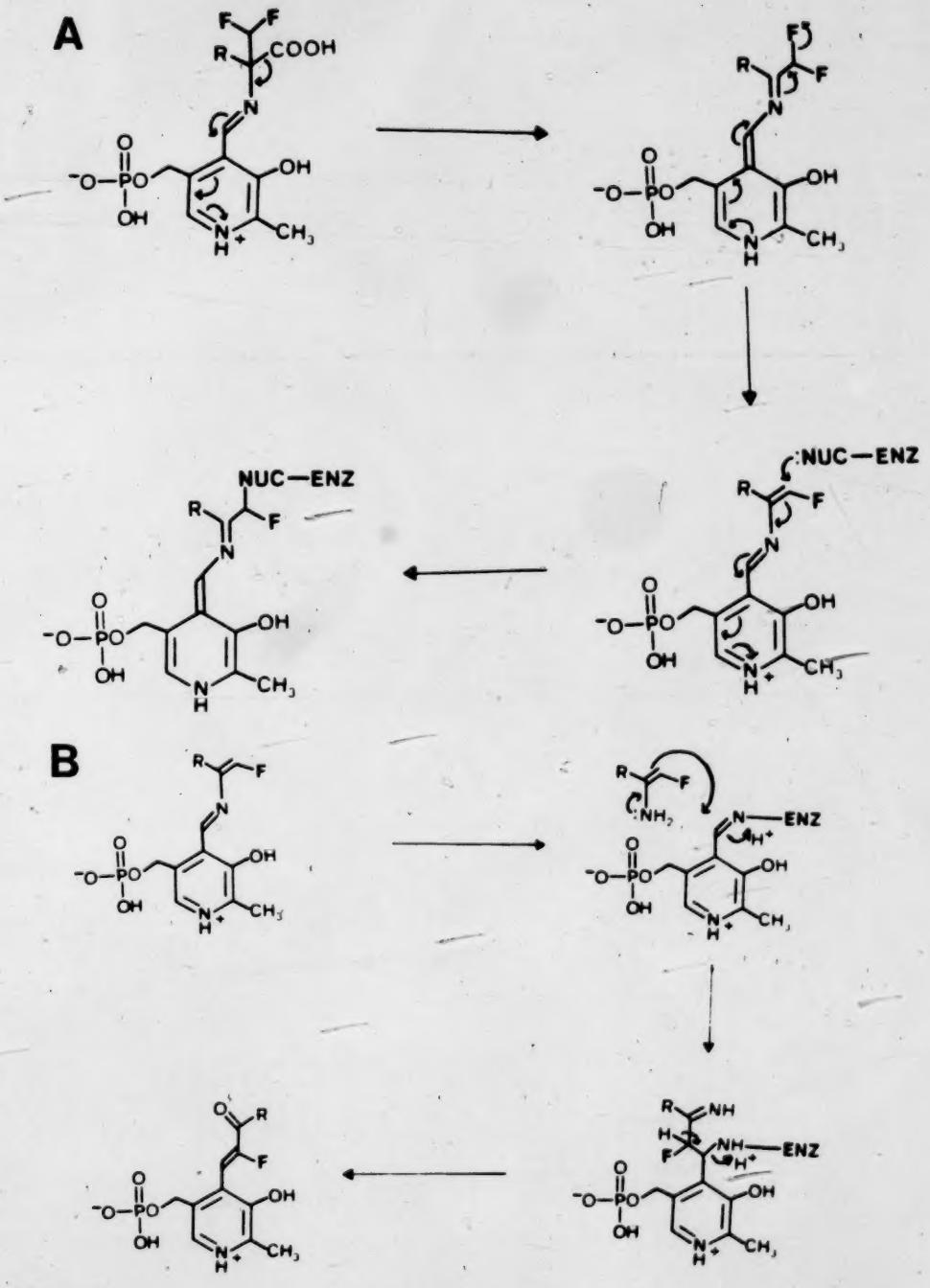


Figure 5. Proposed mechanism of inactivation of pyridoxal phosphate dependent enzymes by fluorinated substrate analogues.

Table 1. Fluorinated inhibitors of pyridoxal phosphate dependent enzymes.

Compound	Enzyme	Reference
β-Fluoroalanine	Alanine racemase	115
	Serine trans-hydroxymethylase	116
ββ-Difluoroalanine	Alanine racemase	117
βββ-Trifluoroalanine	γ-Cystathionase	118
	β-Cystathionase	119
	Tryptophanase	119
	Tryptophan synthase	119
	Threonine dehydrase	119
α-FluoromethylDOPA	DOPA decarboxylase	120
α-DifluoromethylDOPA	DOPA decarboxylase	121
	Aromatic L-amino acid decarboxylase	122,123
α-Fluoromethylhistidine	Histidine decarboxylase	124-129
α-Fluoromethylornithine	Ornithine decarboxylase	130
α-Difluoromethyl ornithine	Ornithine decarboxylase	131,132
α-Fluoromethylglutamic acid	Glutamate decarboxylase	133
α-Difluoromethylarginine	Arginine decarboxylase	134,135
α-Difluoromethyllysine	Lysine decarboxylase	136

N-Hydroxyamino acids are found in nature as components of hydroxamate antibiotics, tumor inhibitors and siderophores[137-142] and as intermediates in the biosynthesis

of cyanogenic glucosides in plants[143-147] and insects.[148] Recently, N-hydroxyglutamic acid has been shown to be an irreversible inhibitor of the pyridoxal phosphate dependent enzymes, glutamate decarboxylase, glutamate-alanine transaminase and glutamate-aspartate transaminase.[149] The proposed mechanism for this inactivation is shown in Figure 6. The N-hydroxy analogue adds to the pyridoxal phosphate in the active site of the enzyme to form a nitrone which is more stable to hydrolysis than the ordinary aldimine intermediate[149] (Figure 4). The nitrone is tightly bound to the enzyme and not released even on dialysis, thereby giving essentially irreversible inhibition.

Hydrazino analogues of amino acids have been known as potent competitive inhibitors of pyridoxal phosphate dependent enzymes for some time (Table 2). These may act by forming hydrazone intermediates (Figure 7) with the pyridoxal phosphate which cannot decarboxylate and are less

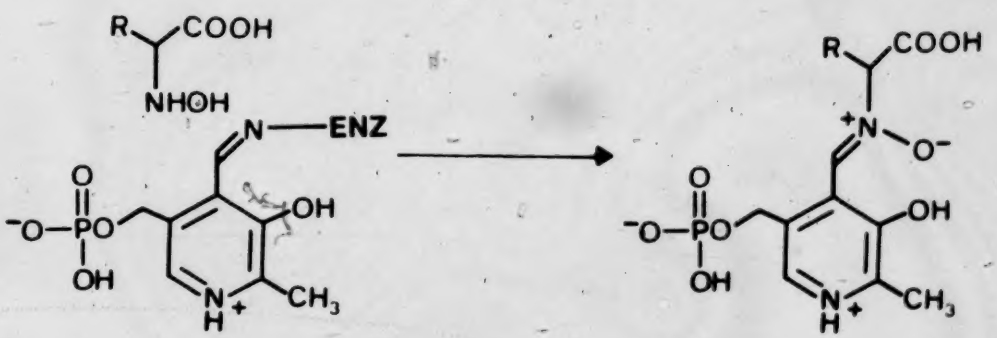


Figure 6. Proposed mechanism of inactivation of pyridoxal phosphate dependent enzymes by N-hydroxyamino acids.

Table 2. Hydrazino analogues of amino acids as inhibitors of pyridoxal phosphate dependent enzymes.

Compound	Enzyme	Reference
N-Aminoaspartic acid	Aspartate aminotransferase	150
N-Aminoornithine	Ornithine decarboxylase	151-153
	Ornithine-keto acid aminotransferase	151
N-Aminohistidine	Histidine decarboxylase	154-155, 129
N-Aminotryptophan	DOPA decarboxylase	156
N-Aminophenylalanine	Aromatic amino acid decarboxylase	157
N-Aminotyrosine	Aromatic amino acid decarboxylase	157
N-AminoDOPA	Aromatic amino acid decarboxylase	157

susceptible to hydrolysis than the ordinary aldimine intermediates (Figure 4). Hydrazino amino acid analogues have been found to inhibit bacterial growth.[158] Linatine, a dipeptide isolated from flax[159] which has been shown to be a wide spectrum antibacterial[160], contains N-amino-D-proline

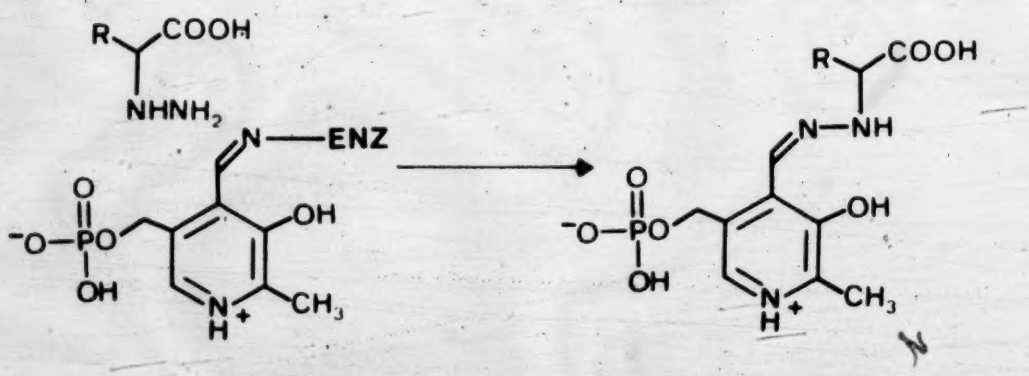
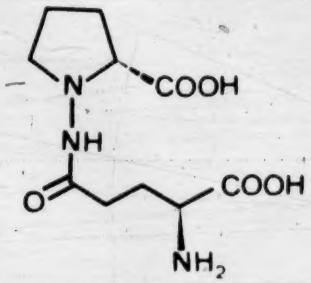


Figure 7. Possible mechanism of inactivation of pyridoxal phosphate dependent enzymes by hydrazino analogues of amino acids.

as its active component. This is the first known natural occurrence of an α-hydrazino acid.



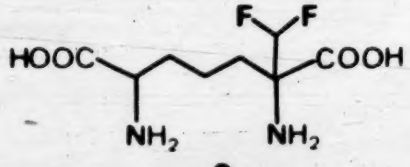
Linatine

There is ample literature precedent to expect that the target diaminopimelic acid analogues **3**, **4** and **5** may be inhibitors of diaminopimelate decarboxylase. To investigate this further, these compounds were synthesized and tested for inhibitory activity against the decarboxylase.

II. Results and Discussion

α-Difluoromethyldiaminopimelic acid

In planning the synthesis of α-difluoromethyldiaminopimelic acid (3), several problems must be considered. Introduction of the difluoromethyl group may be achieved by treating the lithiated anion of an appropriate N-benzylidene amino ester with chlorodifluoromethane by the method of Bey et al.[161] However, as diaminopimelic acid is symmetrical, some means of differentiating between the two ends must be devised to avoid reaction at both. The stereochemistry of the product is also important. meso-Diaminopimelate decarboxylase requires that the substrate diaminopimelic acid have the D or *R* configuration at the end to be decarboxylated while the distal end must have the L or S configuration.[57] Use of the anionic reaction[161] to introduce the difluoromethyl group will make it difficult to control the stereochemistry at that site, although this may not be too important for inhibition of the enzyme.[162] However, control of stereochemistry at least at the distal end is desirable to increase binding of the analogue to the enzyme.



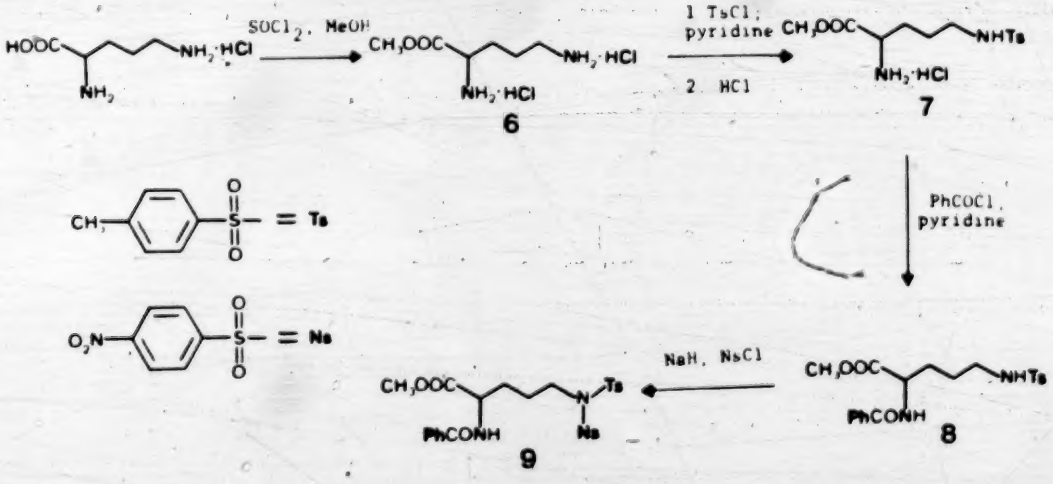
3

The synthesis of 3 can be approached in two ways: piecewise construction of the diaminopimelic acid skeleton

with introduction of a difluoromethyl group at an intermediate stage of the synthesis, or addition of a difluoromethyl group to a suitably protected diaminopimelic acid. The first approach can afford regio- and stereochemical control, but may involve more steps. The second approach has the advantages of simplicity and brevity, but because diaminopimelic acid is a symmetrical molecule, it may be difficult to get reaction exclusively at one end and attempts at monoprotection would be expected to give low yields. Both approaches have been considered and will be discussed.

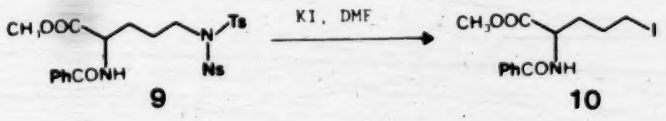
Scheme 1 illustrates the first approach.

Scheme 1



Commercially-available (Sigma) L-ornithine hydrochloride gives the known[163] methyl ester 6 on treatment with thionyl chloride in methanol. The more nucleophilic δ-amino group[164] can be selectively modified with p-toluenesulfonyl chloride

to give the known[165,166] N^5-tosylate 7 whose α-amino group is readily benzoylated to give 8. Reaction with sodium hydride and p-nitrobenzenesulfonyl chloride (NsCl) produces the sulfonimide 9. The next proposed step is the treatment of 9 with potassium iodide by the method of DeChristopher et al[167] to give iodide 10 (equation 1).

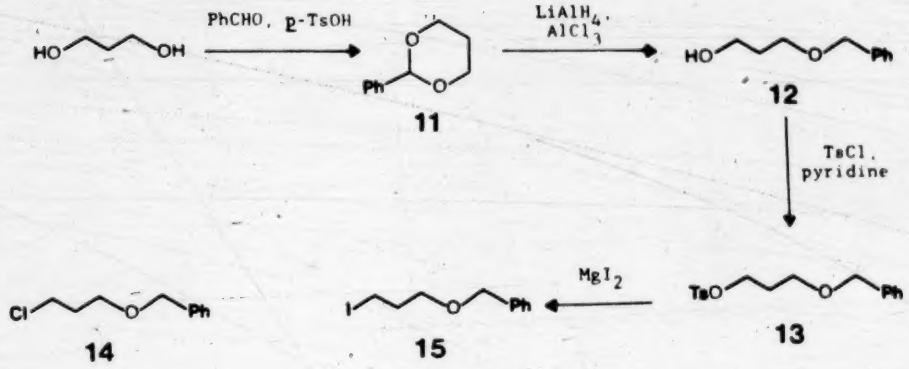


9 10 eq. 1

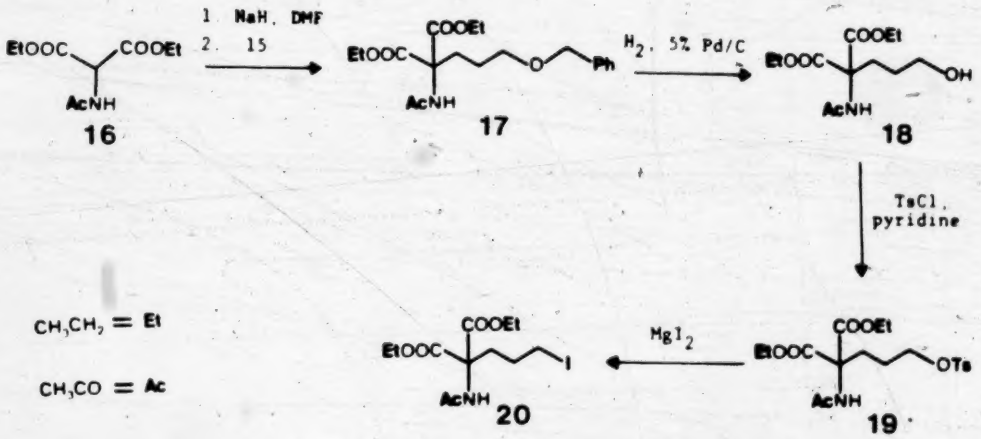
However, this reaction requires prolonged heating in DMF and elimination is a prominent side reaction, even with primary sulfonimides.[167] At this point, more promising routes commanded our attention and this approach was abandoned.

A second attempt involves the use of a 1,3-difunctionalized propane unit which can be selectively manipulated at either terminus (Scheme 2). To this end, the known[168] cyclic acetal 11 was prepared by the procedure of Salmi[169] and was reduced to the alcohol[170] 12. This can be transformed to tosylate[171] 13 and the chloride[172] 14 was a minor (4%) side product in this reaction. Tosylate 13 is readily converted to the iodide[173] 15 with ethereal magnesium iodide by the method of Place et al.[174] This iodide can then be selectively elaborated further (Scheme 3).

Scheme 2

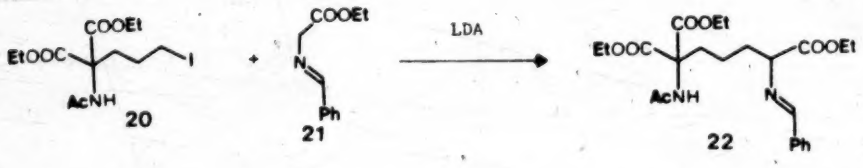


Scheme 3



Treatment of iodide 15 with the sodium salt of diethyl acetamidomalonate[175] (16) gives benzyl ether 17 which is debenzylated by hydrogenolysis to give the known alcohol[176] 18. Reaction with p-toluenesulfonyl chloride gives tosylate 19 which is readily transformed to iodide 20 by magnesium iodide treatment.[174] It was hoped that reaction of 20 with

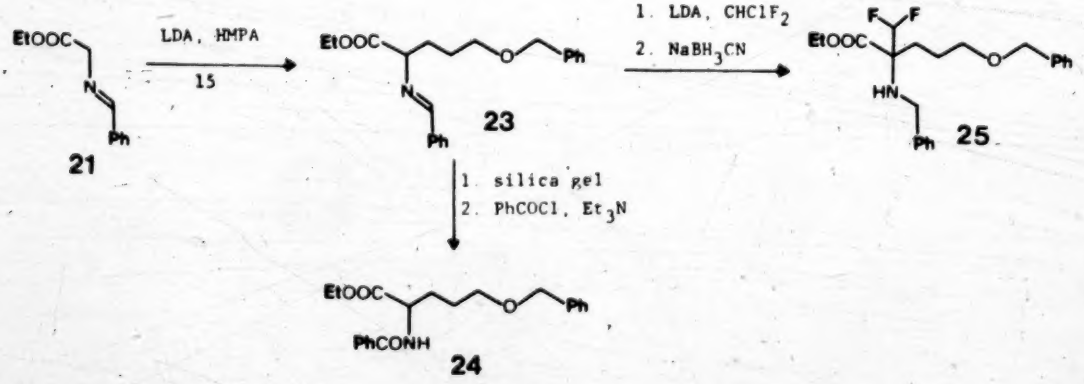
the lithiated anion of ethyl N-benzylidene glycinate[177] (21) would result in the formation of alkylated product 22 (equation 2).



eq. 2

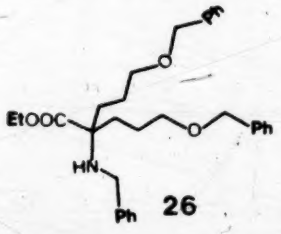
However, a complex mixture resulted from this reaction and no pure product could be isolated even after extensive chromatography. A slightly different approach was attempted to overcome this difficulty (Scheme 4).

Scheme 4

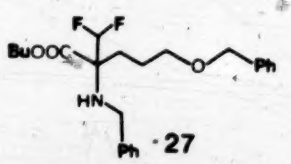


The lithiated anion of ethyl N-benzylidene glycinate[177]

(21) was alkylated with iodide 15 by the method of Stork et al[177] to give imine 23 which was transformed to benzamide 24 for characterization. As imines are relatively unstable and hydrolyse readily on silica gel[177], compound 23 was not purified before the next reaction and this proved to be fortunate. Previous attempts to carry out the difluoromethylation reaction by the method of Bey et al[161] had been unsuccessful. However, the trace of hexamethylphosphoramide (HMPA) remaining in the unpurified imine appeared to catalyse the difluoromethylation reaction, although Bey et al[161] report that the presence of HMPA inhibits the reaction. Thenceforward, a small amount (0.5%) of HMPA was always added to the difluoromethylation reaction mixture. The product imine was immediately reduced to the benzylamino compound 25 with sodium cyanoborohydride.[178] This sequence did not proceed cleanly, however, and several byproducts were formed, two of which could be isolated and identified as the dialkylated derivative 26 and the butyl ester 27.



26



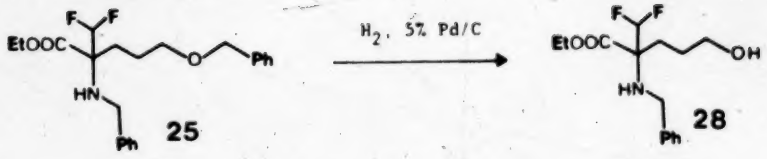
27

$CH_3CH_2CH_2CH_2- = Bu$

The butyl ester 27 probably arises from transesterification of 25 with lithium butoxide present as a contaminant in the butyllithium used to prepare the lithium diisopropylamide. The electron-withdrawing effect of fluorine[179] makes this

reaction more facile.

With **25** in hand, the plan was to prepare the terminal iodide by a sequence similar to that shown in Scheme 3. Before this was attempted, however, it appeared prudent to further protect the benzylamino group to prevent possible intramolecular cyclization at the iodide stage. However, all attempts to acetylate the amine (acetyl chloride/pyridine, acetic anhydride/triethylamine, acetyl chloride/triethylamine, acetic anhydride/*N,N*-dimethylaminopyridine, and acetyl chloride/*N,N*-dimethylaminopyridine) gave primarily recovered starting material or decomposition. It seems that the amine, being both secondary and adjacent to a quaternary center, is too sterically hindered to attack the acetylating agent. The electron-withdrawing effect of the difluoromethyl group[179] may also decrease the nucleophilicity of the amine. In this case, intramolecular cyclization may not be a problem. An attempt was then made to selectively hydrogenolyse the *O*-benzyl group of **25** (equation 3) since *N*-benzyl groups are generally more difficult to hydrogenolyse than *O*-benzyl groups in the absence of acid catalysis.[180] However, a complex mixture of products was obtained, none of which could be identified as the desired alcohol **28**.

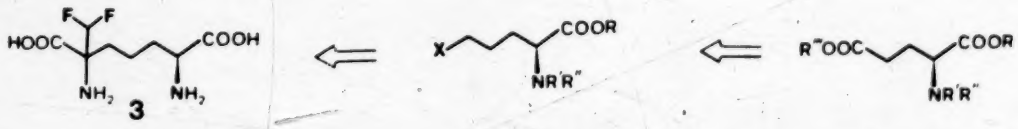


eq. 3

At this point, further study required the preparation of more **25** and because of the problems observed with the route, it was abandoned.

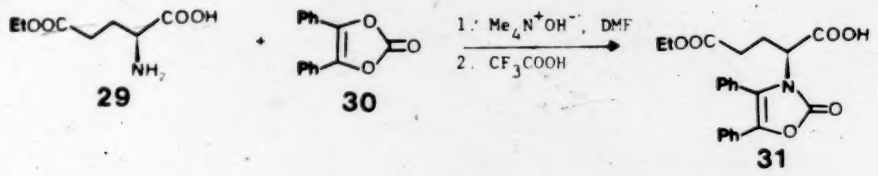
A more stereospecific approach to the piece-wise assembly of **3** is illustrated in Scheme 5.

Scheme 5



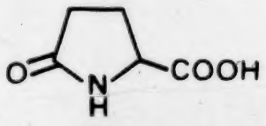
The distal carboxyl group of an optically pure L-glutamic acid derivative can be modified to a leaving group. This can then be reacted with the anion of an N-benzylidene glycinate ester and difluoromethylated to give **3** which has the required *S* stereochemistry at the distal site. Diprotection of the amino group of the glutamic acid synthon is desirable to avoid intramolecular cyclization and the oxazolone group[181] was chosen for this purpose. This is stable to basic and acidic conditions, yet can be removed by hydrogenolysis and gives fluorescent derivatives which are

easily visualized by TLC.[181] Reaction of commercially available (Sigma) 5-ethyl L-glutamate (29) with the stable cyclic carbonate 30[181] by the method of Sheehan and Guziec[181] did not give the expected oxazolone 31 (equation 4).



29 30 31 eq. 4

Instead, intramolecular cyclization[182] occurred under the alkaline reaction conditions to give pyroglutamic acid[183] (32).

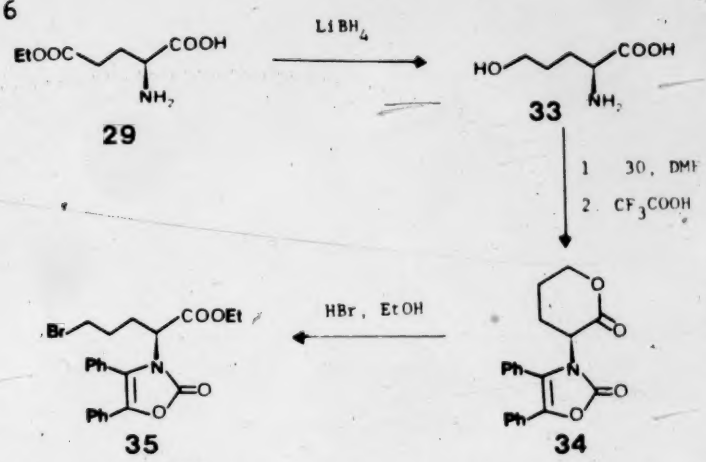


32

To avoid this, 29 was reduced with lithium borohydride by the method of Thompson et al[184] to give alcohol 33[184] (Scheme 6). Alcohol 33 could be protected with 30[181] but it cyclized upon trifluoroacetic acid treatment to give lactone 34. Treatment of 34 with hydrogen bromide in ethanol[185] gives the bromo ester 35.

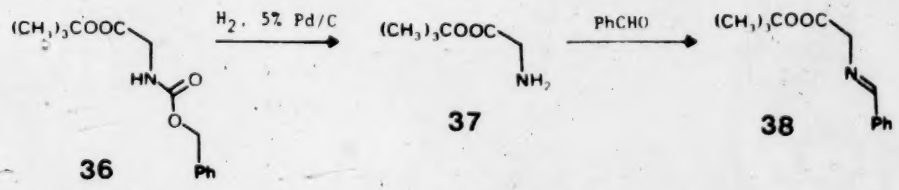
At this point, an N-benzylidene glycine ester was needed for the alkylation. Because of the occurrence of transesterification with the difluoromethyl compound 25, the

Scheme 6



tert-butyl ester[186] 38 was chosen. This would be far less susceptible to nucleophilic attack and is readily removed under acidic conditions.[187] The preparation of 38 is illustrated in Scheme 7.

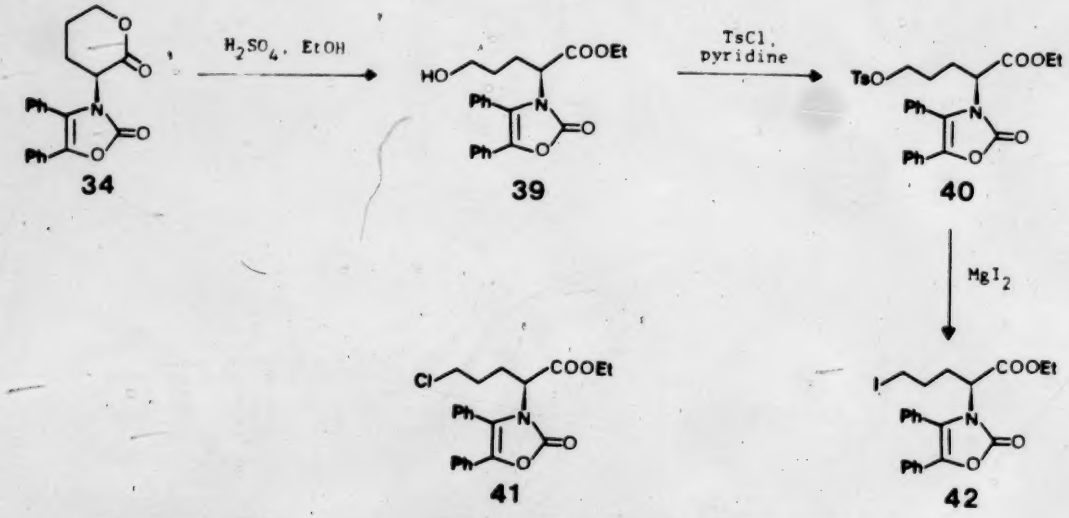
Scheme 7



The known tert-butyl glycinate (37) was prepared via the carbobenzyloxy derivative 36 by the method of Anderson and Callahan.[187] The benzylidene compound 38 was then made by the procedure of Oguri et al.[186] In our hands, this material proved unstable and was destroyed on distillation, contrary to the literature report.[186] However, the freshly-prepared

compound could be stored in the refrigerator for several weeks. With imine 38 in hand, the alkylation reaction[186] could be attempted. Unfortunately, the bromide 35 did not alkylate the lithiated anion of 38 and only decomposition was observed. To study this further, the alkylation of 38 with 1-bromopropane was attempted, but no alkylation was observed. Since it appears that a better leaving group than bromide is necessary, the iodide 42 was prepared (Scheme 8).

Scheme 8

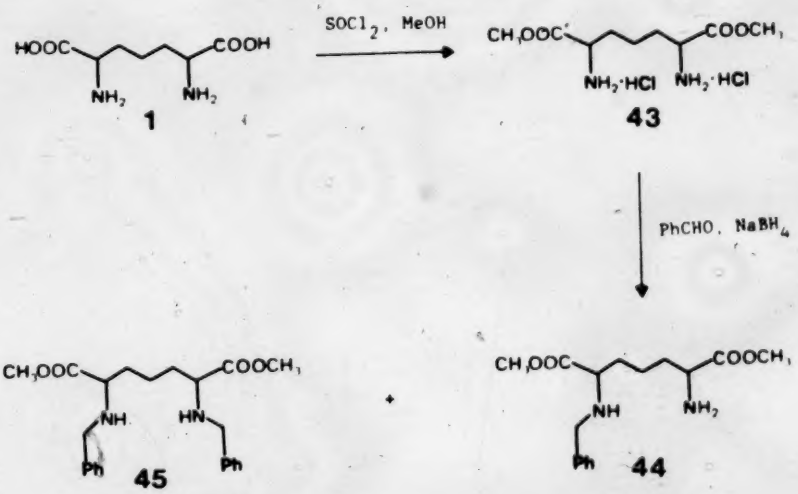


Opening of lactone 34 with ethanol under acidic conditions gives hydroxy ester 39 which reacts with p-toluenesulfonyl chloride to produce tosylate 40. The chloride 41 is a minor (17%) side product. Treatment of 40 with magnesium iodide[174] affords the chromatographically pure but unstable iodide 42. Compound 42 decomposed rapidly, especially when exposed to light, and after a few days in the cold (4°C) and dark, decomposition could be detected.

Alkylation of imine 38 was again attempted with iodide 42, but only decomposition was observed, probably due to the instability of both 38 and 42. Finally, the alkylation of 38 was tried with tosylate 40 but only starting tosylate and decomposition products were observed. This route was then abandoned.

The approach of modifying an existing diaminopimelic acid skeleton was also considered (Scheme 9).

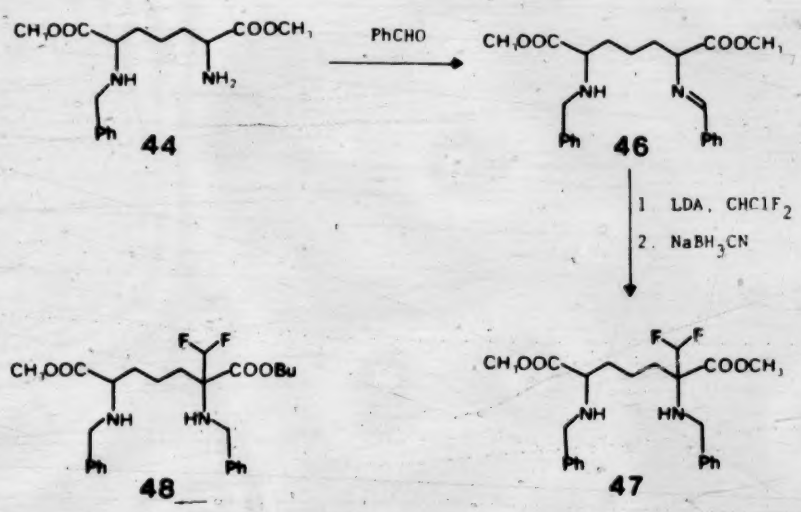
Scheme 9



Commercially available (Sigma) diaminopimelic acid (1) (a mixture of all isomers) was treated with thionyl chloride and methanol to give the known[188,189] dimethyl ester dihydrochloride 43. Reaction of 43 with one equivalent each of benzaldehyde and triethylamine was followed by reduction in situ with sodium borohydride to give a mixture of the desired monobenzylamine 44 (27%) and the dibenzyl compound (45) (18%). It appears that the two reactive centers in 43

are too widely separated to have much influence on each other and little selectivity is observed between mono- and diprotection. The monobenzylamine **44** reacts with benzaldehyde to give imine **46** which was immediately treated with LDA and chlorodifluoromethane[161] in the presence of 0.5% HMPA followed by sodium cyanoborohydride reduction to give the difluoromethyl compound **47** in 3% yield (Scheme 10).

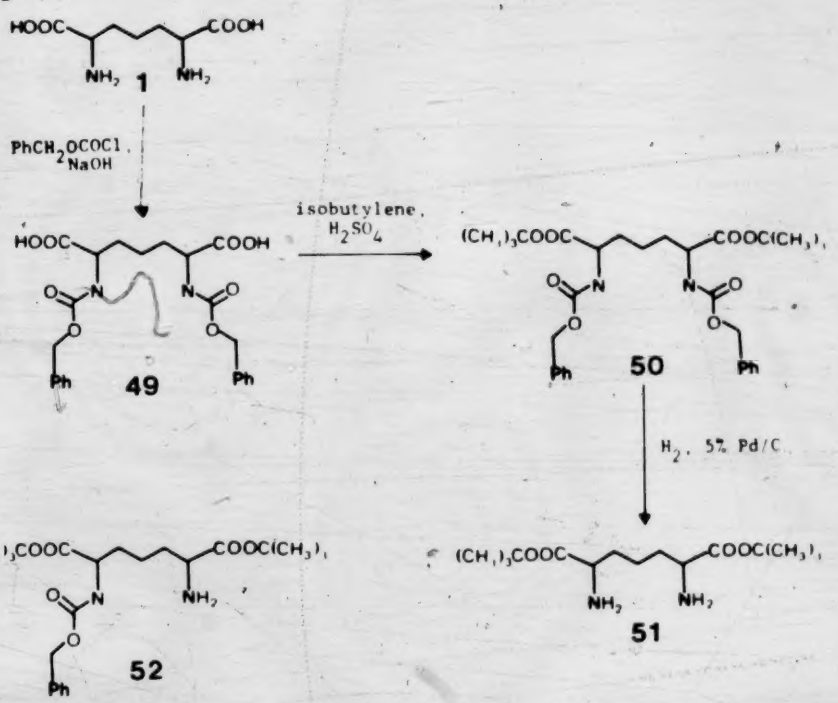
Scheme 10



The monobutyl ester **48** was also isolated in 5% yield. These compounds were not very stable and repeated chromatography was necessary to isolate them from the complex reaction mixture. Again, the transesterification problem was observed, due to the fluorine-induced increased electrophilicity[179] of the nearby ester. This may account in part for the large number of products observed and the low stability of compounds **47** and **48**. To avoid this problem, the more sterically hindered tert-butyl ester **51** was used

(Scheme 11).

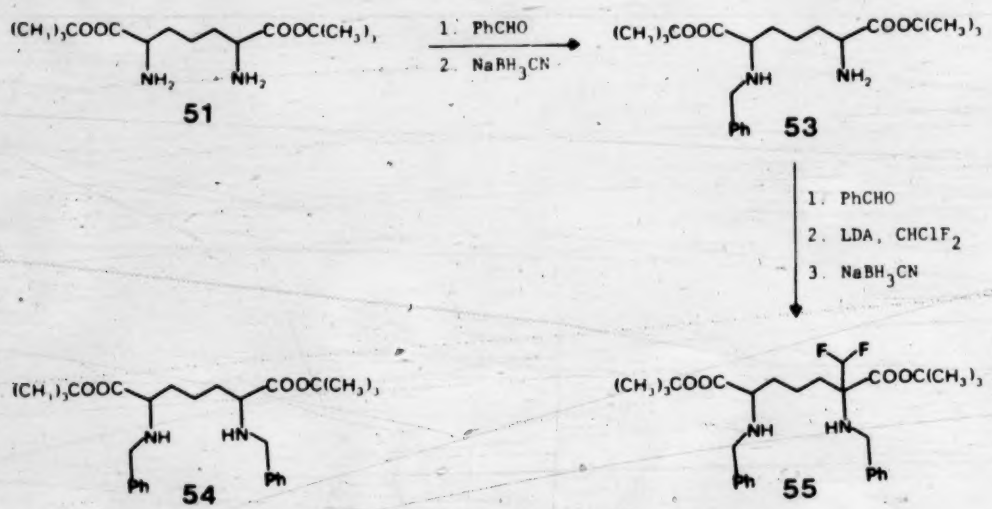
Scheme 11



Commercial diaminopimelic acid (1) was treated with benzyl chloroformate and aqueous sodium hydroxide by the method of Wade et al[190] to give the dicarbobenzyloxy derivative **49**.[190] The meso isomer can be separated from the racemate at this stage by recrystallization[188,190] but, as stereochemical scrambling will occur in the later stages of the synthesis, the mixture of isomers was carried through. The di-tert-butyl ester **51**[191] was prepared from **49** via the N-protected ester **50**.[191] The course of the hydrogenolysis of **50** was very dependent on the batch of catalyst used and a small quantity (15%) of the monocarbobenzoxy derivative **52** could be isolated. This could be increased to 30% by

performing the hydrogenolysis at atmospheric pressure and monitoring the formation of 52 by TLC. The di-tert-butyl ester 51 was treated with one equivalent of benzaldehyde, followed by sodium cyanoborohydride[178] to give both the monobenzylamine 53 (34%) and the dibenzyl amine 54 (33%) (Scheme 12).

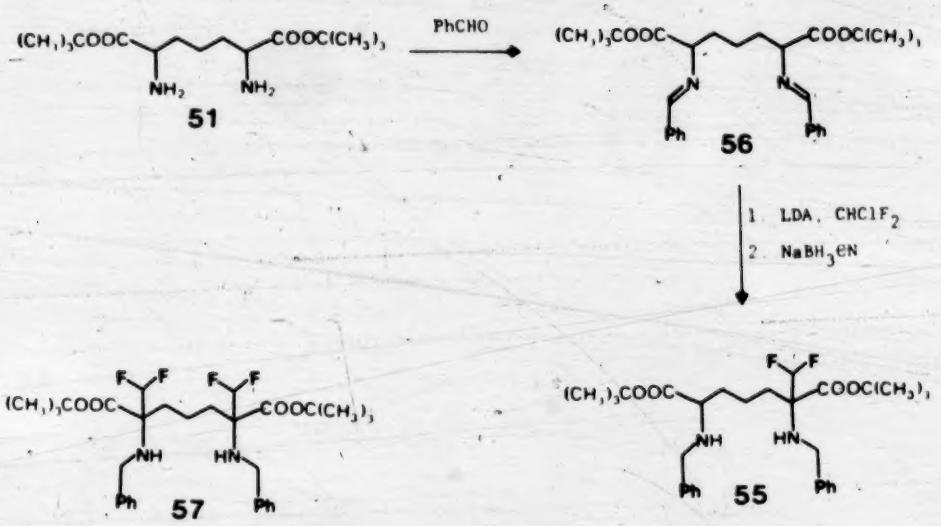
Scheme 12



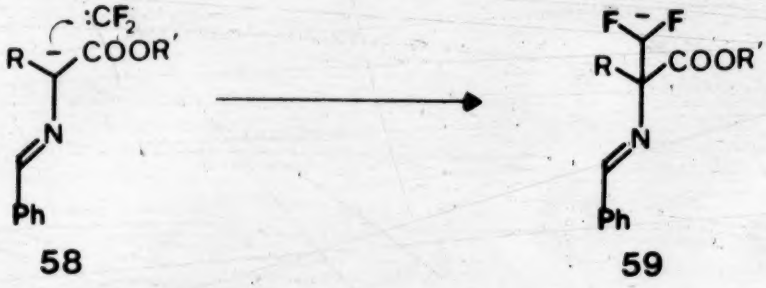
The monobenzyl compound 53 was treated with benzaldehyde, difluoromethylated[161] and reduced to give compound 55 in 26% yield. This is an improvement over the reaction with dimethyl ester 44, but the formation of a number of unidentified side products is still observed. Low yields in both the monobenzylation and difluoromethylation steps and the length of the route prompted examination of the diimine 56 which was prepared from 51 (Scheme 13).

Compound 56 might be expected to react with one equivalent of LDA to give a monoanion which would react with

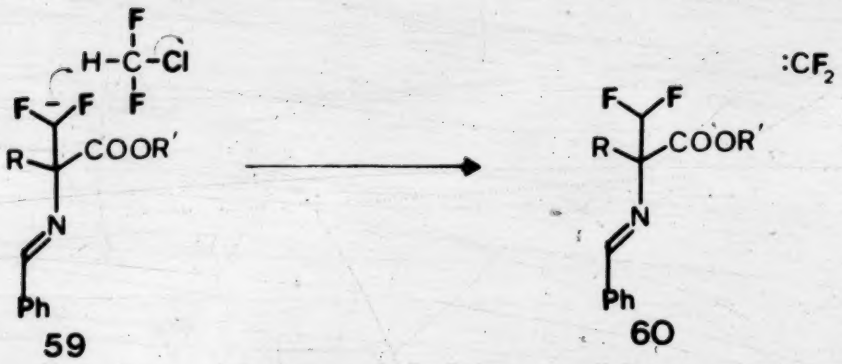
Scheme 13



chlorodifluoromethane to give predominantly the mono(difluoromethyl) derivative **55**. In practice, both **55** (16%) and the bis(difluoromethyl) compound **57** (25%) were produced. Bey *et al*[161] propose that this reaction proceeds *via* the reactive difluorocarbene (equations 5-6).

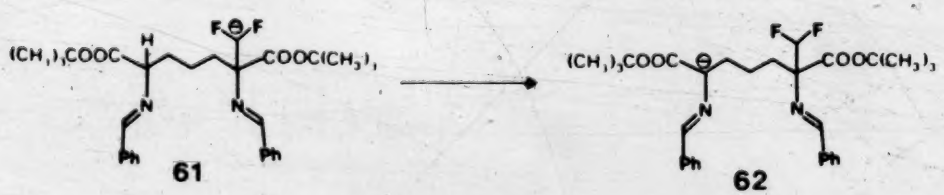


eq. 5



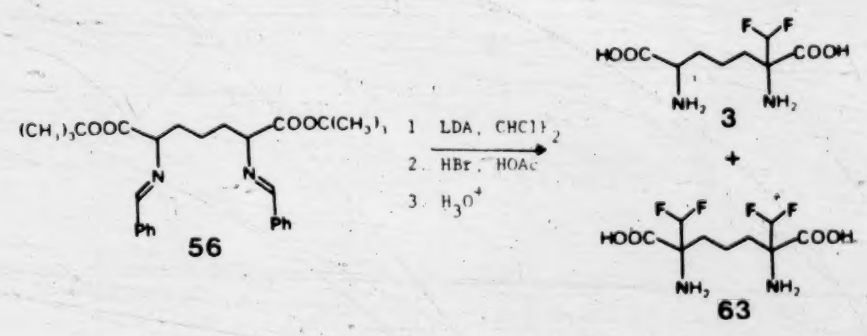
eq. 6

The difluorocarbene which might be initially produced by the action of the imine anion **58** or excess LDA on chlorodifluoromethane can react with anion **58** to give difluoroanion **59**. This can abstract a proton from another molecule of chlorodifluoromethane to generate product **60** and difluorocarbene which can continue the chain process. The presence of the reactive difluorocarbene may account for some of the many unidentified side products in this reaction. Also, the anion **61** of compound **55**, once formed in the reaction mixture, may abstract a proton intramolecularly rather than from a chlorodifluoromethane molecule, to give anion **62** which can lead to the bis(difluoromethyl) product **57** (equation 7).



eq. 7

In the interest of shortening the route yet further, the intermediate difluoromethyl imines were hydrolysed[161,187] directly to the difluoromethyl amino acids 3 and 63 as mixtures of stereoisomers. Compounds 3 and 63 could be separated and purified by ion exchange chromatography and were obtained in 15% and 9% overall yields respectively (equation 8).



eq. 8

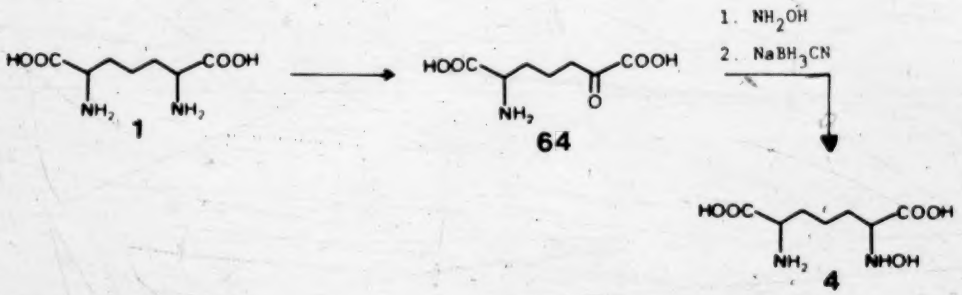
With the target difluoromethyl analogue 3 in hand, testing for inhibition of meso-diaminopimelate decarboxylase and antibacterial activity could be performed. These results are given in section III.

N-Hydroxydiaminopimelic acid

The synthesis of N-hydroxyamino acids has been accomplished by several methods including the reaction of α-bromo acids or esters with sodium anti-benzaldoximate[192-196], the oxidation of amino acids[197,198], and the reduction of oximes of α-keto acids.[149,199-200] The last approach was initially chosen for the synthesis of N-hydroxydiaminopimelic acid 4. It first appeared that

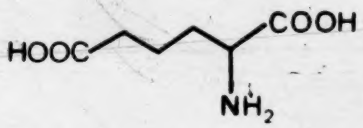
enzymatic reaction of diaminopimelic acid (1) with an amino acid oxidase or dehydrogenase would form the α-keto acid 64 or its corresponding cyclic imine which could then react with hydroxylamine and sodium cyanoborohydride[178] by Ahmad's procedure[199] to give 4 (Scheme 14).

Scheme 14



Amino acids with charged side groups, such as lysine, glutamic acid and aspartic acid are not good substrates for snake venom L-amino acid oxidase (Sigma) or hog kidney D-amino acid oxidase (Sigma)[201] and no significant conversion was observed when diaminopimelic acid was treated with either of these enzymes. Work[202] reported that diaminopimelic acid reacts even more slowly with snake venom L-amino acid oxidase than does lysine. However, meso-diaminopimelate-D-dehydrogenase from Bacillus sphaericus[47-51] specifically catalyses the oxidation of meso-1 at the D center to give 64 and has the advantage that, as the LL and DD isomers are not substrates[50], the commercially available mixture of isomers can be used directly. This enzyme was isolated from B. sphaericus[49] by

Dr. M.M. Palcic (Department of Food Science, University of Alberta) and allowed to react with diaminopimelic acid by the procedure of White[47] with regeneration of the expensive NADP cofactor or, better, by the procedure of Misono et al.[48] Unreacted diaminopimelic acid was removed by ion exchange chromatography[48] and the crude ninhydrin-active material was treated immediately with hydroxylamine hydrochloride. The only isolated product, however, was α-aminoadipic acid[203] (65).

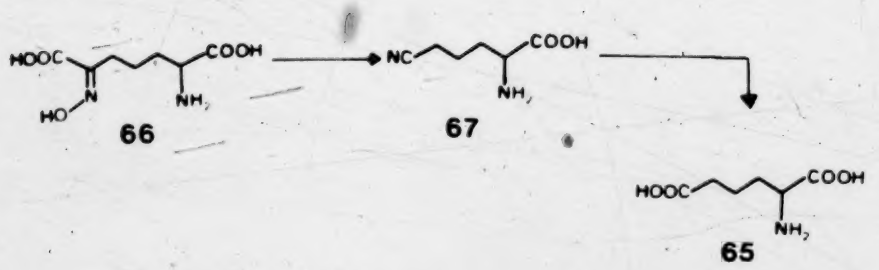


65

There are two possible explanations for this. It is known that α-keto acid oximes are converted to nitriles on warming in aqueous solution and that the reaction is acid-promoted[204] (equation 9).

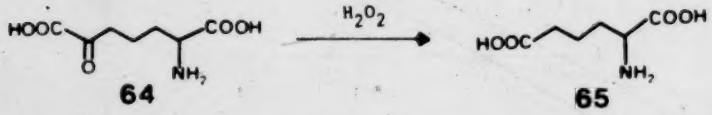
eq. 9

One possibility is that oxime 66 formed from α-keto acid 64 was decarboxylated to α-aminoadiponitrile 67 and further hydrolysed to 65 (equation 10).



eq. 10

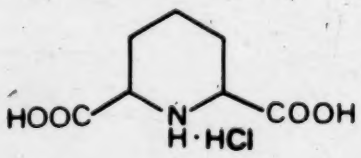
However, it does not seem likely that hydrolysis of the nitrile to an acid would occur under the mild conditions used (2 M hydrochloric acid, room temperature, 24 h). Nitriles generally require stronger conditions for hydrolysis, such as heating with concentrated acid or base.[205] For example, the N-carbobenzyloxy derivative of nitrile 67 required 25 h reflux in 4 M HCl for its hydrolysis.[203] An alternative explanation is that hydrogen peroxide is generated in the enzymic system and reacts with 64 to give 65 directly (equation 11).



eq. 11

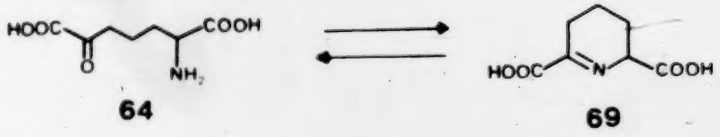
Misono et al[48] have observed that the product of the dehydrogenase reaction reacts with hydrogen peroxide to give α-aminoadipic acid. This appears to be the more likely

explanation, as α-aminoadipic acid was observed chromatographically as the only ninhydrin-active product in another dehydrogenase reaction mixture not treated with hydroxylamine. Addition of catalase to the reaction mixture prevented the formation of α-aminoadipic acid. The reaction was repeated, including catalase in the mixture to decompose any hydrogen peroxide formed. Removal of the unreacted diaminopimelic acid by ion exchange chromatography[48] gave a new ninhydrin-active material which was incubated with hydroxylamine hydrochloride and sodium cyanoborohydride.[199] It was hoped that any oxime formed would be reduced in situ before decarboxylation could occur to the nitrile.[204] However, the only product isolated was piperidine-2,6--dicarboxylic acid which was characterized as its hydrochloride salt[206] (68).



68

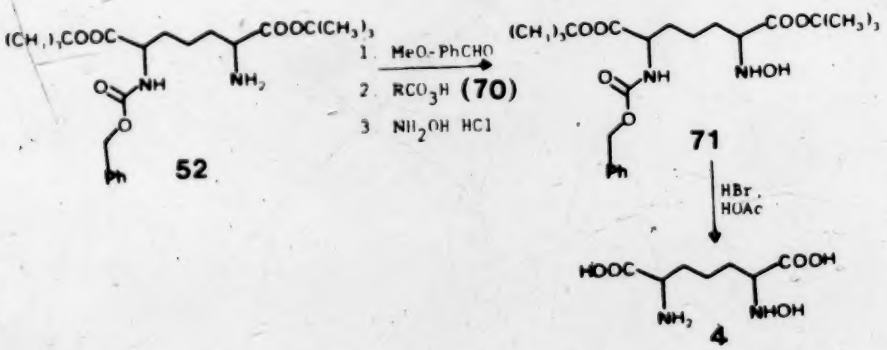
α-Keto acid 64 exists in equilibrium with its cyclized form 69 (equation 12) and this was apparently reduced before oxime formation could occur.



eq. 12

It appeared another approach was necessary. The oxidation methodology of Polonski and Chimiak[198] was chosen, as the monoprotected diaminopimelic acid derivative 52 was already available as a mixture of isomers (Scheme 11). The route followed is shown in Scheme 15.

Scheme 15



Monoamine 52 was treated with p-methoxybenzaldehyde, oxidized with monoperphthalic acid[208] (70) and deprotected without purification of intermediates to give hydroxylamine 71. Deprotection with hydrogen bromide in acetic acid[187]

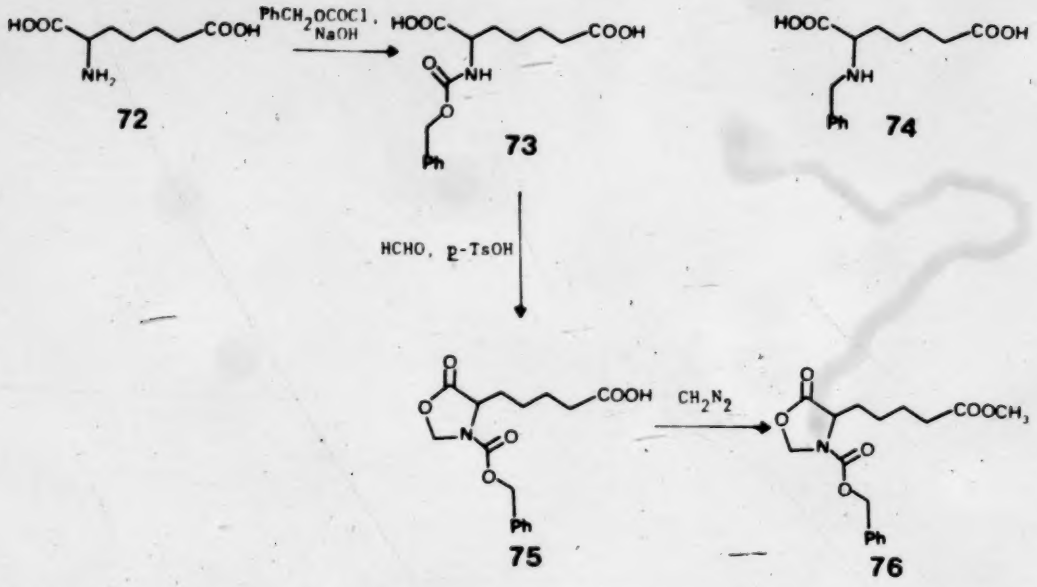
gave the chromatographically-pure N-hydroxydiaminopimelic acid **4** as a mixture of isomers which rapidly reduced Fehling's solution.[192,209] The results of testing of **4** for decarboxylase inhibition and antibacterial activity are shown in section III.

N-Aminodiaminopimelic acid

The synthesis of α-hydrazino carboxylic acids has been accomplished by many methods, including the reaction of α-halo acids with hydrazine[210-213], the reduction of hydrazones of α-keto acids[156,214], the oxidative amination of amino acids[215-218] and reaction of aldehydes with hydrazine and cyanide in a Strecker approach.[219,220] Recently, an alternative methodology involving the attack of lithium enolates on dialkyl azodicarboxylates to form α-hydrazino acids has been developed in our laboratory.[221] Since α-aminopimelic acid (**72**) is commercially available (Sigma), anionic amination of a suitably protected derivative of **72** would afford easy access to the N-aminodiaminopimelic acid skeleton (Scheme 16).

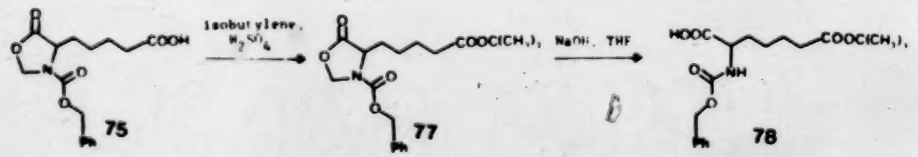
Treatment of **72** with benzyl chloroformate and sodium hydroxide gives the known[222] carbobenzyloxy derivative **73** as well as the benzyl compound **74** as a side product. Compound **73** reacts with paraformaldehyde by the method of Itoh[223] to give the oxazolidone **75**. Treatment of **75** with diazomethane or methyl chloroformate and methanol[224] gives methyl ester **76**. However, attempts to quench the lithium anion of **76** with

Scheme 16

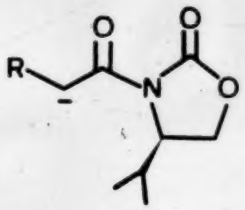


dibenzyl azodicarboxylate or with acetic acid led only to decomposition. Since the oxazolidone moiety of 76 appeared too unstable to survive anion formation conditions, it was removed by treatment with sodium hydroxide in aqueous THF.[225] Unfortunately, the methyl ester was also removed under these conditions and diacid 73 was isolated. To avoid these difficulties, the tert-butyl ester 77 was prepared as this should be much less susceptible to basic hydrolysis (Scheme 17).

Scheme 17



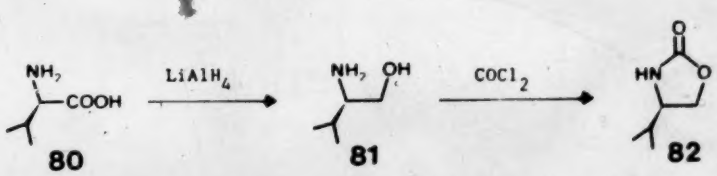
Treatment of acid 75 with isobutylene by the method of Itoh[223] gives the tert-butyl ester 77, although in poor yield (21%). The oxazolidone moiety is readily cleaved under basic conditions[225] to leave acid 78. However, an attempt to form and quench the lithiated trianion of 78 with dibenzyl azodicarboxylate failed, giving only recovered starting material.



79

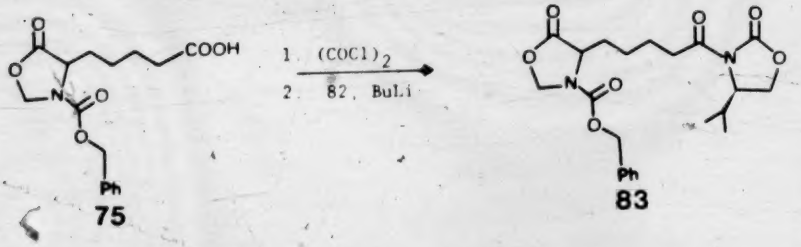
It is known that the α-anions of acylated chiral oxazolidones 79 react efficiently and stereospecifically[226] with many electrophiles including dibenzyl azodicarboxylate.[221] In adapting this approach to the present problem, the *R* oxazolidone was chosen as this would give the required *R* configuration[226] at the hydrazino center. Preparation of the chiral oxazolidone[226,227] 82 was accomplished by phosgene treatment of *R*-valinol 81, available by reduction of D-valine[228] (80) (Scheme 18).

Scheme 18



80 81 82

Treatment of the acid chloride formed by reaction of 75 with oxalyl chloride[229] with the lithium salt of 82 resulted in the formation of acyl oxazolidone 83 (equation 13).

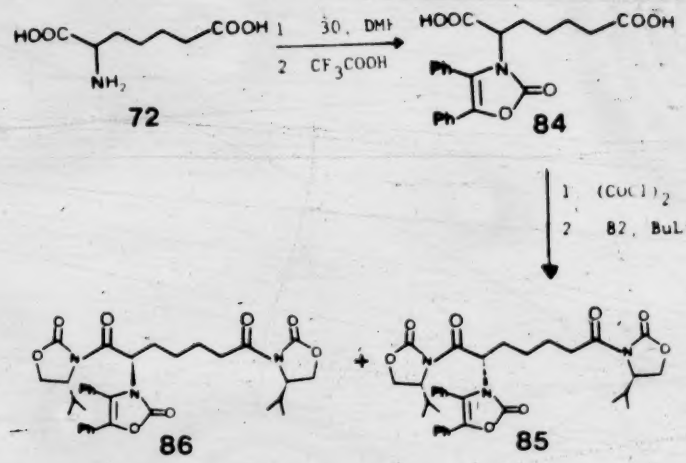


eq. 13

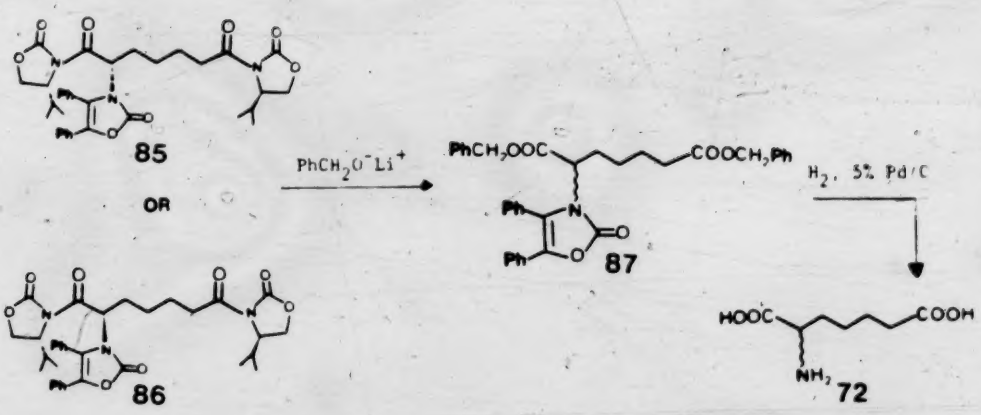
Due to the instability of the carbobenzyloxyoxazolidone moiety to base, it was thought prudent to remove this and reprotect with a different group before attempting anion formation. However, treatment of 83 with aqueous sodium hydroxide in THF[225] caused loss of both oxazolidone moieties and gave diacid 73. At this point, the use of the carbobenzyloxyoxazolidone was discontinued.

Since diprotection of the amino group of the α-aminopimelic acid skeleton is probably necessary to avoid interference of a nucleophilic nitrogen atom in the amination reaction, the amino group was protected using the method of Sheehan and Guziec[181] to give diacid 84 (Scheme 19). Treatment of 84 with oxalyl chloride[229] followed by reaction of the crude diacid chloride with two equivalents of the lithium salt of 82 gave the diastereomers 85 and 86 which could be separated by flash chromatography.[230] In an attempt to identify these isomers, (Scheme 20) both 85 and

Scheme 19

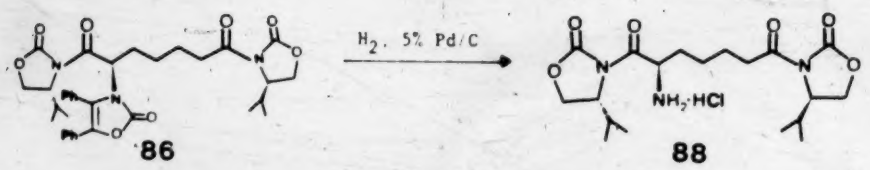


Scheme 20



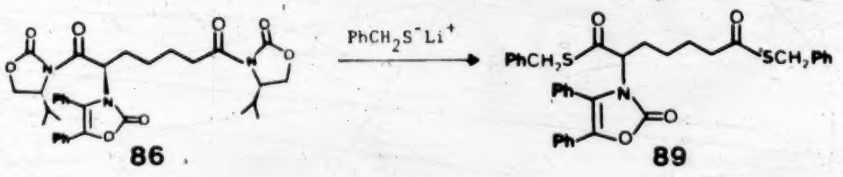
86 were separately treated with lithium benzyloxide[226] to give samples of the dibenzyl ester 87 with very low optical rotation values. Upon hydrogenolysis of 87 derived from either 85 or 86, α-aminopimelic acid 72 was obtained which had essentially no optical activity. It appeared that racemization was occurring during the benzyloxide treatment,

despite the report of Evans et al[226] that this can be done with no loss of stereochemical integrity. However, the oxazolone protecting group is very bulky and can hinder the attack of benzyloxide on the adjacent carbonyl. As the benzyl ester is slowly formed, excess benzyloxide is still present in the mixture to catalyse racemization. Several attempts to overcome this deprotection problem failed. Hydrogenolysis of 86 gives the optically active amine hydrochloride 88 (equation 14) but attempts to hydrolyse the acyl oxazolidones with aqueous base gave mixtures of optically inactive products.



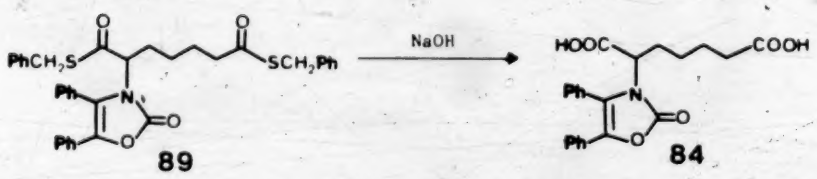
86 88 eq. 14

It seems that while basic hydrolysis of an unsubstituted acyl oxazolidone such as 83 is facile, even a small α-substituent like an amino group hinders the reaction enough for racemization and hydrolysis of the chiral oxazolidone itself[226] to occur. Cleavage of the oxazolidone groups of 86 with lithium benzylthiolate occurs rapidly and cleanly to give the dithioester 89 (equation 15).



86 89 eq. 15

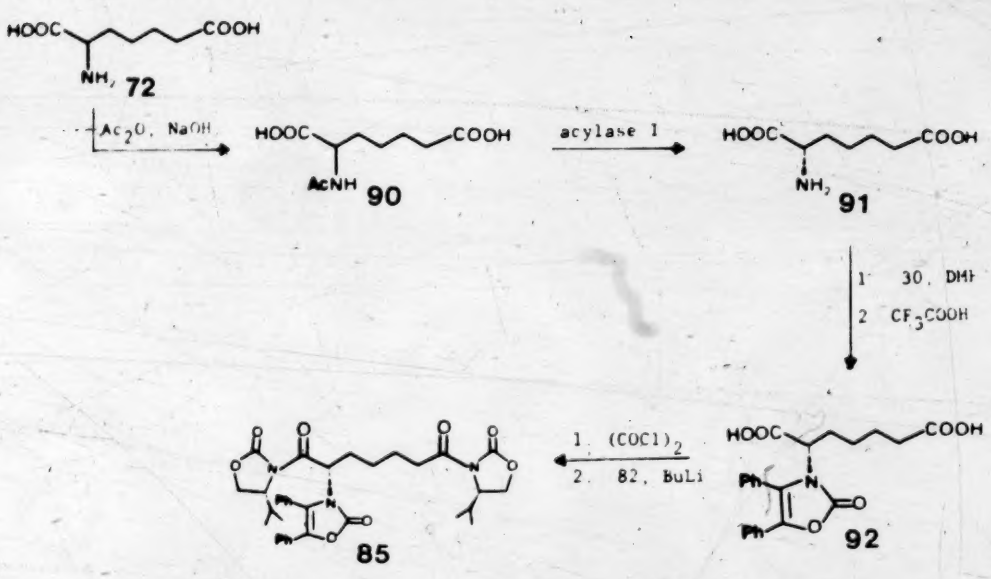
However, basic hydrolysis of 89 was unexpectedly[231] slow and the isolated diacid 84 was optically inactive (equation 16). An attempt to use silver ion-catalyzed hydrolysis[232] gave no hydrolysis product.



89 84 eq. 16

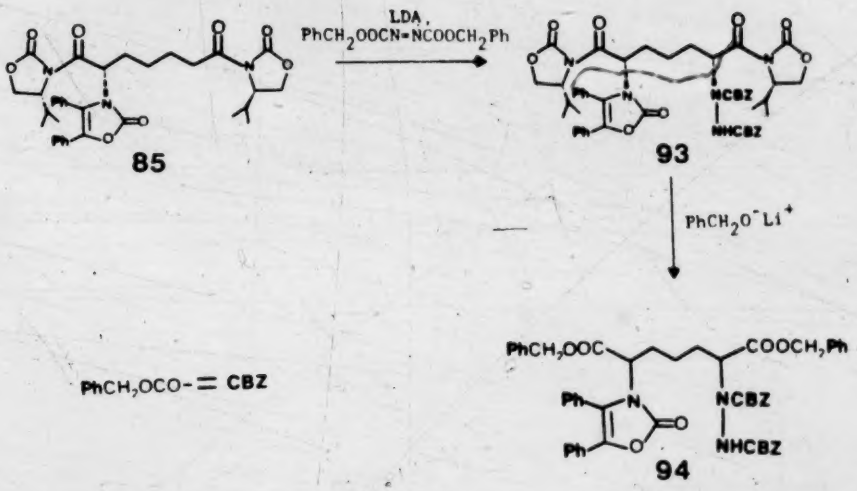
The identification of 85 and 86 was eventually accomplished by the preparation of material of known stereochemistry (Scheme 21). L-α-Aminopimelic acid (91) was prepared by the method of Wade et al[190] via the acetamide 90[190] and was transformed to the oxazolone 92 by the method of Sheehan and Guziec.[181] Upon treatment of the acid chloride of 92 with the lithium salt of 82, 85 was obtained. No trace of the isomer 86 could be detected.

Scheme 21

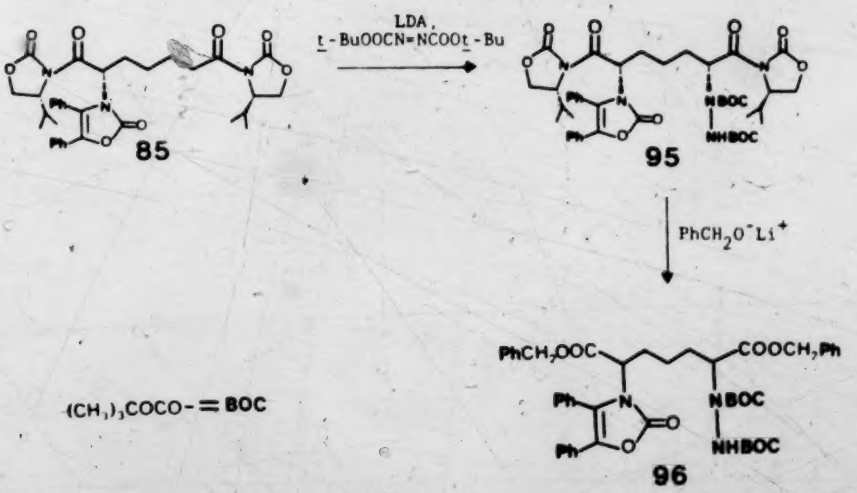


Treatment of **85** with LDA and dibenzyl azodicarboxylate gives the hydrazino derivative **93** (Scheme 22). Interestingly, **86** fails to react under these conditions and can be recovered from the reaction mixture. Compound **93** is transformed to dibenzyl ester **94** upon treatment with lithium benzyloxide[226]. The bis(oxazolidone) **93** gives broad peaks in the NMR, due to the presence of rotomers, but on warming the NMR solution in deuterotoluene to 100°C, sharp peaks are seen, and the compound appears >95% stereochemically pure. However, the dibenzyl ester **94** shows no measurable optical activity and is presumably a mixture of stereoisomers due to base-catalysed epimerization by analogy with formation of **87**. Compound **85** also reacts with LDA and di-tert-butyl azodicarboxylate[233] to give adduct **95** (Scheme 23) which again appears stereochemically clean by elevated temperature NMR.

Scheme 22

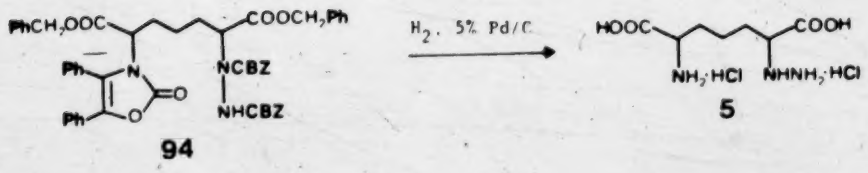


Scheme 23



However, when 95 is treated with lithium benzyloxide, optically inactive 96 is formed, giving no improvement. Derivative 94 was then hydrogenolysed to give the

dihydrochloride salt of N-aminodiaminopimelic acid 5 as a mixture of stereoisomers (equation 17).



eq. 17

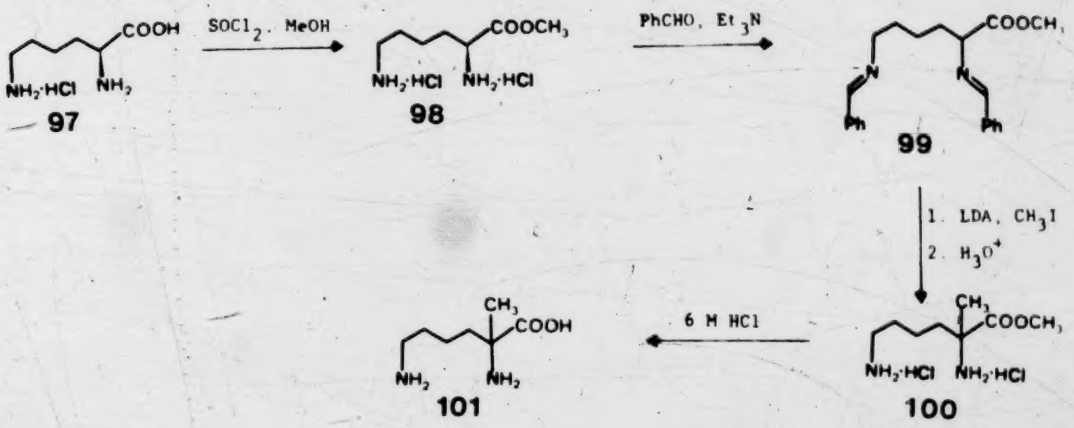
Compound 5, while apparently chromatographically pure, is very hygroscopic and rapidly becomes gummy on exposure to air. Hydrazines are known to absorb CO_2 and water rapidly from the air.[234] Compound 5 was tested for inhibition of the decarboxylase and antibacterial activity and the results are shown in section III.

α-Methyl Lysine

A meso-diaminopimelate decarboxylase inhibitor will prevent a pathogen from making its own lysine, but will show reduced effectiveness as an antibiotic if the pathogen can ingest lysine from the exogenous pool. Methylated analogues have been shown[103] to inhibit the active transport of amino acids and, to this end, α-methyl lysine was prepared (Scheme 24).

L-Lysine hydrochloride (97) upon treatment with thionyl chloride and methanol gives the known[235,236] methyl ester

Scheme 24



98. The diimine 99[161] was prepared by the method of Bey et al[161] and its lithiated anion was alkylated with methyl iodide.[237] Mild acid hydrolysis[161] gives the methyl ester 100 and the known[238] α-methyl lysine (101) is obtained by further hydrolysis in refluxing 6 M hydrochloric acid.

The α-methyl lysine was tested by Dr. M.A. Pickard[239] (Department of Microbiology, University of Alberta) for inhibition of lysine transport[96-101] in Escherichia coli and Bacillus sphaericus. At concentrations 10- and 100-fold larger than that of lysine, uptake of radioactively labelled lysine was inhibited 20-50% in both bacteria, but even at concentrations equimolar to that of lysine, uptake was not significantly inhibited.[239]

Stereochemistry of the meso-Diaminopimelate Decarboxylase Reaction.

In preparation for the testing of the diaminopimelic acid analogues 3, 4, and 5 as inhibitors of meso-

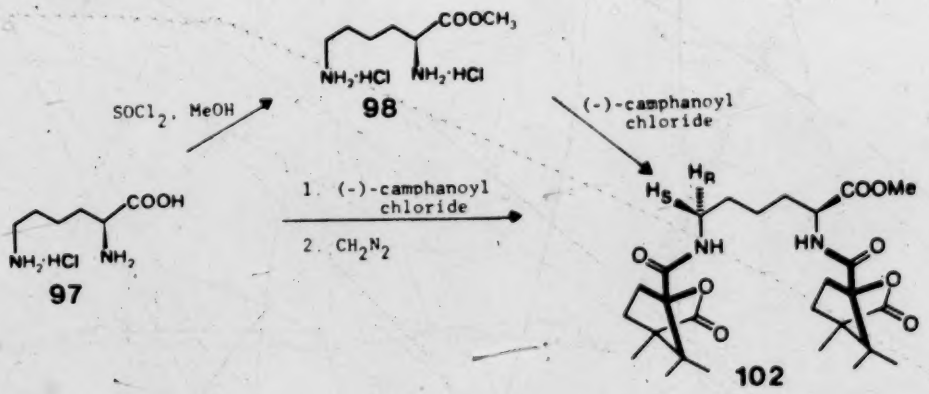
diaminopimelate decarboxylase, the enzyme was isolated by Dr. M.M. Palcic (Department of Food Science, University of Alberta) from two sources: Bacillus sphaericus[57] and wheat germ[37] (Triticum vulgaris). It had been reported[64] that the enzyme from B. sphaericus catalyses the decarboxylation of diaminopimelic acid to lysine with an unusual inversion of configuration, in contrast to the retention mode observed for all other pyridoxal phosphate dependent α-decarboxylases studied.[56,104-106,240-249] This unique finding led us to attempt to determine whether the enzyme from a eukaryotic source (wheat germ) behaves in the same way, and to confirm the previous report.[64]

The determination of the stereochemical course of an α-decarboxylase-catalysed reaction is generally performed so that the product is labelled at the decarboxylation site with two different isotopes of hydrogen, one of which comes from the solvent medium to replace the departing carboxyl group. The problem then becomes the determination of the configuration of this labelled methylene group. This problem has been approached in several ways[56,104-105] and usually involves correlating the product with a compound of known stereochemistry by a series of enzymatic and chemical reactions. This was the approach taken by Asada et al.[64] NMR methods have also been used, including direct ^{1}H NMR[241] and ^{1}H NMR of camphanamide derivatives of the product amines using europium shift reagents.[242-243,247] Recently, a new NMR technique for observing the position of deuterium in a

stereospecifically-deuterated methylene group has been developed[250], namely use of either selective or normal two-dimensional ^{1}H-^{13}C NMR shift correlation spectroscopy[251-254] with deuterium decoupling. This technique was employed in determining the stereochemical course of the B. sphaericus and wheat germ meso-diaminopimelate decarboxylase reactions.

Methyl L-lysinate[235,236] 98 which was prepared from L-lysine (97) by treatment with methanol and thionyl chloride was transformed to the biscamphanamide 102 by reaction with (-)-camphanoyl chloride (Scheme 25).

Scheme 25

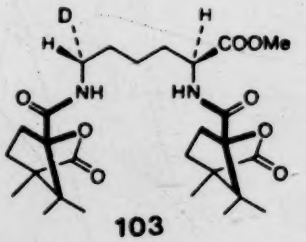


Alternatively, L-lysine (97) can be treated with (-)-camphanoyl chloride by the procedure of Armarego et al[255], followed by diazomethane to give 102; this procedure was followed for the derivatisation of the deuterium

labelled compounds. The biscamphanamide was chosen, as precedent[256] showed that in such compounds, the diastereotopic hydrogens on the methylene group adjacent to nitrogen give well resolved ^{1}H NMR signals in deuterobenzene solution. As well, the signal due to the pro-*S* hydrogen is always downfield from that of the pro-*R* hydrogen. In biscamphanamide **102**, the two diastereotopic hydrogens resonate at δ 3.26 and δ 3.11 and these signals correlate to the ^{13}C NMR signal which appears at δ 38.8. The C-6 methylene portion of the ^{1}H-^{13}C shift correlation map of **102** is shown in Figure 8.* The ^{13}C spectrum is shown along the horizontal axis and the ^{1}H spectrum, along the vertical axis (δ increases right to left).

Diaminopimelic acid (a mixture of **DD**, **LL** and meso isomers) was incubated with the B. sphaericus meso-diaminopimelate decarboxylase in D_2O and the resulting lysine was isolated and derivatised with camphanoyl chloride and diazomethane to give **103**. Methyl camphanate[257] (**104**) was also isolated as a byproduct.

104



103

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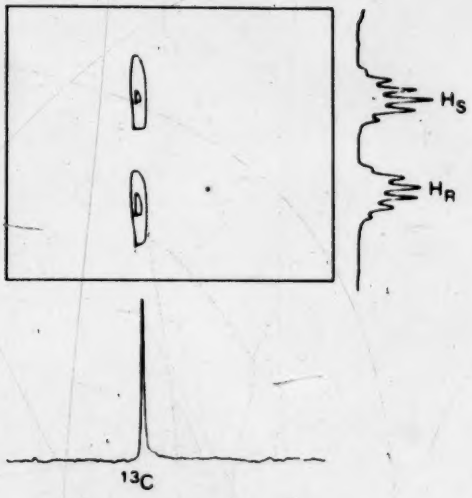

Figure 8. C-6 Methylene region of the shift correlation map of 102.

The C-6 methylene region of the deuterium decoupled ^{1}H-^{13}C shift correlation map of a mixture of 103 and unlabelled 102 is shown in Figure 9D.* As in Figure 8, the ^{13}C spectrum is shown along the horizontal axis and the ^{1}H spectrum along the vertical axis. The downfield ^{13}C signal arises from C-6 of the unlabelled 102 and correlates to both proton signals, as in Figure 8. However, C-6 bearing a deuterium, from 103, resonates upfield, due to the α-deuterium isotope effect.[258-259] This signal correlates to only the most downfield proton resonance, corresponding to H_S[256] at C-6. This shows that the pro-*R* position of the C-6 methylene group is occupied by deuterium, and inversion of

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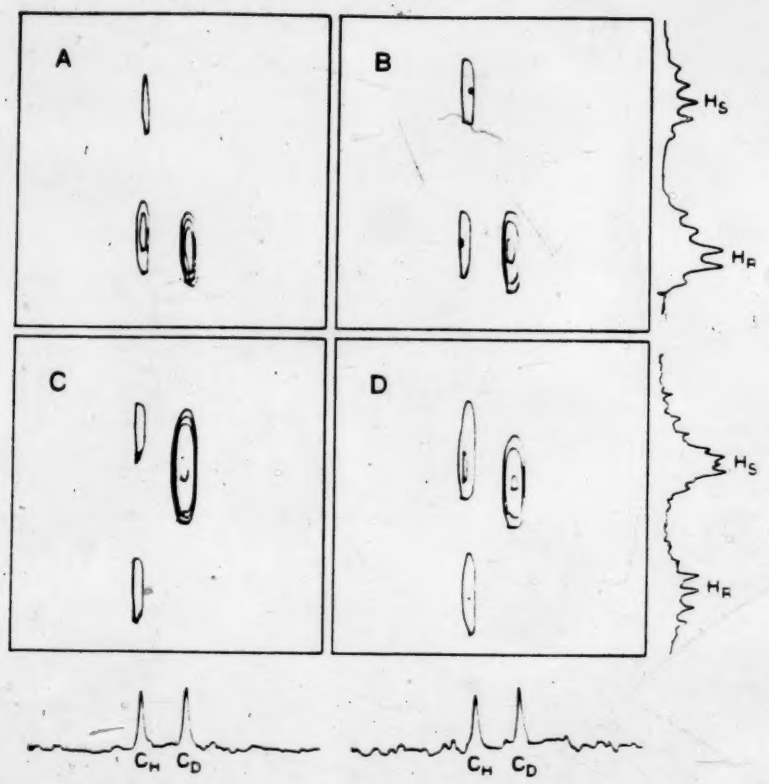
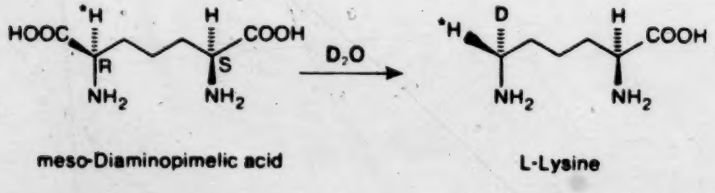


Figure 9. C-6 Methylene regions of the deuterium decoupled shift correlation maps of **106** (A and B) and **103** (C and D) diluted with **102**.

configuration occurs[64] in the decarboxylase reaction (equation 18).



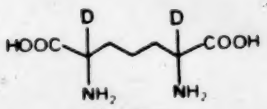
meso-Diaminopimelic acid L-Lysine

eq. 18

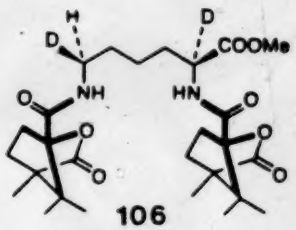
It is interesting to note that this ^1H NMR signal is shifted slightly upfield with respect to the corresponding signal

from the unlabelled compound. This may arise from a deuterium induced β-isotope shift.[260]

To provide confirmation of this, [2,6-$^2H_2$]-diaminopimelic acid[64] (105) was prepared by the method of Fujihara and Schowen[261] as a mixture of isomers and incubated with the B. sphaericus decarboxylase . Reaction of the meso isomer gave lysine which was isolated and derivatised to give the biscamphanamide 106.



105



106

The deuterium decoupled ^1H-^{13}C shift correlation map of a mixture of 106 and unlabelled 102 (Figure 9B) shows that the ^{13}C signal arising from the deuterated C-6 of 106 correlates only to the upfield H_R signal, implying that the pro-S position is now deuterated. This confirms the results of Asada et al[64] that the B. sphaericus meso-diaminopimelate decarboxylase reaction proceeds with inversion of configuration.

The decarboxylase isolated from wheat germ was subjected to the same experiments. When incubated with diaminopimelic acid in D_2O, the resulting lysine gave compound 103 on derivatisation as before. This showed an identical deuterium decoupled ^1H-^{13}C shift correlation

spectrum (Figure 9C) to that of the lysine biscamphanamide obtained from the B. sphaericus meso-diaminopimelate decarboxylase reaction (Figure 9D). The complementary experiment, wherein [2,6-$^{2}H_{2}$]-diaminopimelic acid 105 was incubated with the wheat germ enzyme in D_2O, gave lysine which was derivatised to biscamphanamide 106. The deuterium decoupled ^{1}H-^{13}C shift correlation spectrum of this (Figure 9A) was identical to that of the lysine biscamphanamide obtained from the B. sphaericus reaction (Figure 9B). The results demonstrate that the wheat germ meso-diaminopimelate decarboxylase reaction also proceeds with inversion of configuration.

In many pyridoxal phosphate dependent enzyme catalysed reactions, reaction occurs on one face of the planar pyridoxal phosphate-substrate complex.[262,263] The bond to be broken in the substrate is aligned perpendicular to the π-electron system of the complex[56,105,264] so that effective orbital overlap can occur. In the case of decarboxylases, this is the C_{α}-COOH bond. The incoming proton is then expected to assume the same position as did the leaving carboxyl, giving the retention of configuration observed with most decarboxylases.[56,104,105] However, this cannot be the case with meso-diaminopimelate decarboxylase from either B. sphaericus or wheat germ as inversion of configuration is observed, and the incoming proton must attach to the face opposite to that from which the carboxyl group departed

(Figure 10*).

Two explanations have been suggested.[64] The incoming proton may be donated from a group in the active site on the opposite side of the complex to that from which the carboxyl group leaves and this group may be reprotonated by solvent later in the catalytic cycle. Alternatively, a large conformational change[265-266] in the complex may occur to expose its opposite face to solvent protons once the carboxyl group has departed. In any case, the similarity in the stereochemical course of the reaction of both the B. sphaericus and wheat germ meso-diaminopimelate decarboxylase implies a similarity in their mechanism. It is possible that these enzymes share a common evolutionary pathway which is convergent to that of other α-decarboxylases.

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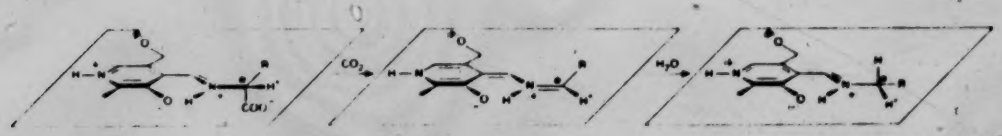
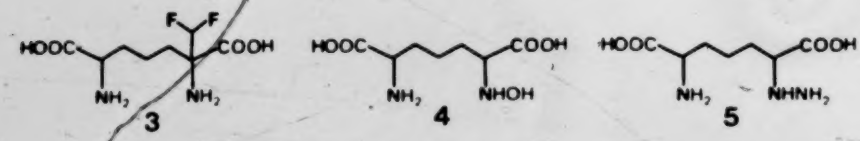


Figure 10. Possible orientation of the substrate-cofactor complex in meso-diaminopimelate decarboxylase.

III. Biological Studies

Enzyme Inhibition

The testing and kinetic studies of diaminopimelic acid analogues 3, 4 and 5 were done in collaboration with Dr. M.M. Palcic.



Assays for direct competitive inhibition of meso-diaminopimelate decarboxylase were performed by monitoring the release of $^{14}CO_2$ from [1,7-^{14}C] diaminopimelic acid in the presence and absence of the test analogue. Time-dependent irreversible inhibition was also studied by incubating the analogue with enzyme and measuring any loss of activity after various time intervals.

Surprisingly, the α-difluoromethyl analogue, (3) not only was ineffective as a suicide inhibitor but was not even a good competitive inhibitor. At a concentration of 0.6 mM, 3 did not inhibit the wheat germ enzyme and even at 10 mM concentrations, only 30-35% inhibition of the B. sphaericus enzyme was observed. The bis(difluoromethyl) analogue (63) had about the same potency, inhibiting the B. sphaericus enzyme 60% at a concentration of 11 mM. This is probably a consequence of the strict steric requirements of meso-diaminopimelate decarboxylase. Since even the DD and LL

isomers of diaminopimelic acid are neither substrates nor inhibitors of the enzyme[57], it is likely that the difluoromethyl group introduces too much steric bulk to allow 3 to enter the active site.

Both compounds 4 and 5 were strong inhibitors of meso-diaminopimelate decarboxylase, however. The N-hydroxy analogue 4 shows a K_i value of 0.71 mM with the wheat germ enzyme and 0.91 mM for the B. sphaericus decarboxylase. In the case of the hydrazino analogue 5, the K_i value is 0.084 mM for the wheat germ enzyme and 0.10 mM for the B. sphaericus enzyme. The K_m values for diaminopimelic acid are 0.16 mM for the enzyme from wheat germ[37] and 1.7 mM for the B. sphaericus decarboxylase.[57] Both inhibitors are competitive and show no time dependent irreversible inhibition.

These analogues were also tested as inhibitors of meso-diaminopimelate-D-dehydrogenase. Assay of enzyme activity was performed by monitoring NADPH formation spectrophotometrically at 340 nm. Both direct competitive and time-dependent irreversible inactivation were studied, as in the case of the decarboxylase. Neither 3 nor 4 caused any significant inhibition of this enzyme, and in a direct competitive assay, 5 showed no activity, either. However, in a preliminary experiment, 5 did exhibit time-dependent inhibition when incubated with the enzyme in the absence of substrate and this inhibition was slowly reversed under the assay conditions. It appears that 5 is a slow-binding

inhibitor of the enzyme and does not compete effectively with substrate for the active site, but once in the active site, is hard to displace. Further studies are in progress now to investigate this behaviour.

Antibacterial Activity

The analogues 3, 4 and 5 were tested for antibacterial activity by Dr. M.A. Pickard. The difluoromethyl analogue 3 did not inhibit bacterial growth in liquid medium when present at a concentration of 400 μg/mL (*E. coli*, *Bacillus subtilis*, and *B. cereus*) or on plates when applied on disks (200 μg) (*Arthrobacter simplex*, *Micrococcus roseus* and *M. lysodeikticus*). This is not unexpected in light of the enzyme inhibition results. Neither the *N*-hydroxy analogue 4 nor the hydrazino analogue 5 showed growth inhibitory activity in complex nutrient broth when tested at concentrations of up to 100 μg/mL against *E. coli*, *B. subtilis*, *B. cereus*, *B. megaterium*, *Staphylococcus aureus* or *Streptomyces antibioticus*. However, in defined media, both 4 and 5 inhibited the growth of *B. megaterium* 75% at concentrations as low as 20 μg/mL and the growth of *B. subtilis* almost completely at 500 μg/mL. *E. coli* and *Pseudomonas aeruginosa* were not affected. The lack of effect in complex media may be due to bypass of the blockage of lysine synthesis by ingestion of exogenous lysine. It is also possible that the analogues are not readily ingested by the bacteria and therefore do not have a chance to exert an

effect. This problem may be overcome by incorporating the analogues into small peptides that can enter the bacteria more readily and be hydrolysed inside the organism to release the potential antibiotic.[267-269] Payne and coworkers[267-269] have successfully used this approach to increase the antibacterial activity of aminooxy and hydrazino analogues of amino acids. Further studies are in progress to prepare and test such peptides.

Chapter 2

Fast Atom Bombardment Mass Spectra of Nucleosides and Nucleotides

I. Introduction

Mass spectrometry possesses great potential as a technique for study of biological molecules because only small amounts of sample are required of materials which may be available only in minute quantities.[270] However, in the past, its use has been hindered by the involatility of large polar molecules and by their sensitivity to the extreme conditions needed to bring them into the gas phase.[271] The advent of milder desorption ionization methods, including fast atom bombardment, has solved this problem for many types of naturally occurring molecules.[272]

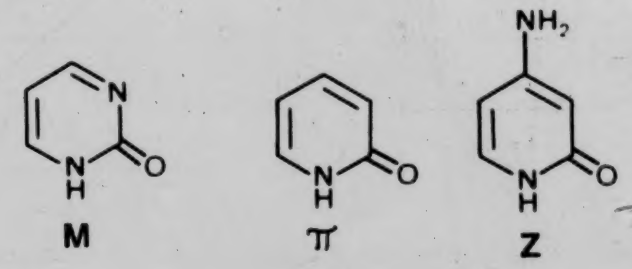
Fast atom bombardment mass spectrometry (FAB MS)[273-277] is a technique whereby a stream of fast atoms (typically argon or xenon) impinges onto a solution of the sample to be analysed in a liquid matrix such as glycerol. Volatilization and ionization[278] of the analyte occurs and the resulting ions, either positive or negative, can be detected in the usual way. The "sputtering" or desorption of the analyte into the gas phase is most efficient when the fast atom beam strikes the surface of the glycerol at approximately a 20° angle.[279-282] Several reviews have been published on the use of this technique.[279-282]

The study of nucleotides by mass spectrometry has been difficult because of the involatility and thermal instability of these compounds. However, recently, other "soft" ionization methods[283-289] such as field desorption[290-294], californium-252 plasma desorption[295-298],

secondary ion mass spectrometry[299-304], pulsed laser-induced desorption[305-306] and atmospheric pressure ionization mass spectrometry[307] as well as pyrolysis electron impact and chemical ionization mass spectrometry[308-313] have been applied successfully in this area. These methods have also been used to study the adducts of nucleosides with psoralens[314] and dehydroretronecine.[315] These reports indicate that FAB MS should be a useful tool in the study of nucleosides and nucleotides, especially considering the simplicity of the technique and the ease with which existing mass spectrometers are converted to its use.[316] Not only might molecular weight be routinely determined, but, if fragmentation of oligonucleotides occurs between the sugar and phosphate residues of the backbone, sequence information might be obtained. This would provide a rapid alternative to chemical and enzymatic sequencing procedures.[317-319] Also, protected intermediates in the synthesis of oligonucleotides[320] might be analysed more easily[321], as well as nucleosides and nucleotides containing unnatural base residues.[322]

Since this work was begun, several publications have appeared reporting the use of FAB MS in the study of free[323-330] and protected[331-332] nucleosides and nucleotides. Nucleoside adducts with carcinogens[333-334], cyclic nucleotides[335] and chromium and cobalt complexes of nucleoside triphosphates[336] have also been studied by this technique. In collaboration with Dr. C. Tamm (Institute for

Organic Chemistry, University of Basel), we decided to investigate the use of FAB MS as a tool for the characterization of nucleosides and nucleotides containing the cytosine analogues 2(1H)-pyrimidinone (M), 2(1H)-pyridinone (π) and 4-amino-2(1H)-pyridinone (Z).[337-340]

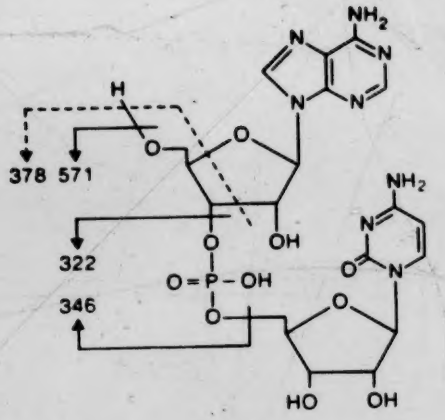


In the following discussion, the nomenclature XpYp... will be used where X, Y, etc. are the usual nucleoside symbols (A, adenosine; T, thymidine; U, uridine; C, cytidine; G, guanosine) and p represents a phosphate group. The nucleoside X is at the 5' end of the oligonucleotide. In the case of protected nucleosides, ϕ represents a p-chlorophenyl group and CE, a cyanoethyl group.

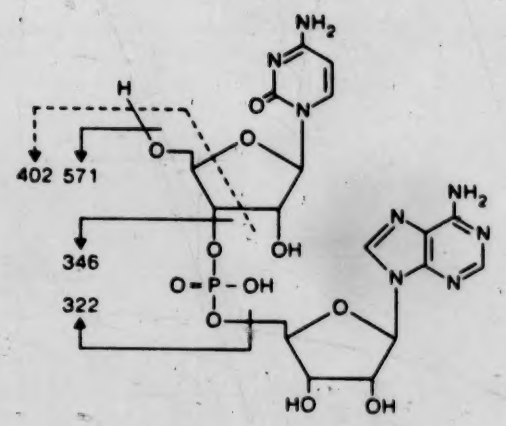
II. Results and Discussion

In preparation for the study of these unnatural nucleotides, several commercially-available (Sigma) dinucleotides in both the ribo and deoxyribo series were studied by negative ion FAB MS. Quasimolecular ion ($M-H^-$) peaks were seen in each case, as well as those corresponding to glycerol-solvated quasimolecular ions. The major mode of fragmentation is cleavage of the sugar-phosphate linkages to give nucleoside monophosphate anions. Very little, if any cleavage of the base-sugar bond was observed, in accord with the results of Eagles et al[324], although Sindona et al[330] reported significant fragmentation of the base. However, due to the high background below m/z 260, spectra were generally not recorded below this value, and thus any peaks due to free base anions would not be observed. In nearly all cases, fragmentation of the 5'-sugar residue occurred, giving peaks corresponding to the 3'-end nucleotide plus a C_3H_4O fragment. This pattern was also noted by Crow et al.[323] This allows discrimination between the 5'-end and 3'-end nucleotides, and identification of the direction of the sequence. This is illustrated in Figure 11 for the isomeric dinucleotides ApC and CpA.

Trinucleotides were also examined by negative ion FAB MS. Again, quasimolecular ions and glycerol-solvated quasimolecular ions were seen. The expected mono- and dinucleotide fragments, as well as the diphosphorylated central nucleoside fragment unambiguously assign the



ApC



CpA

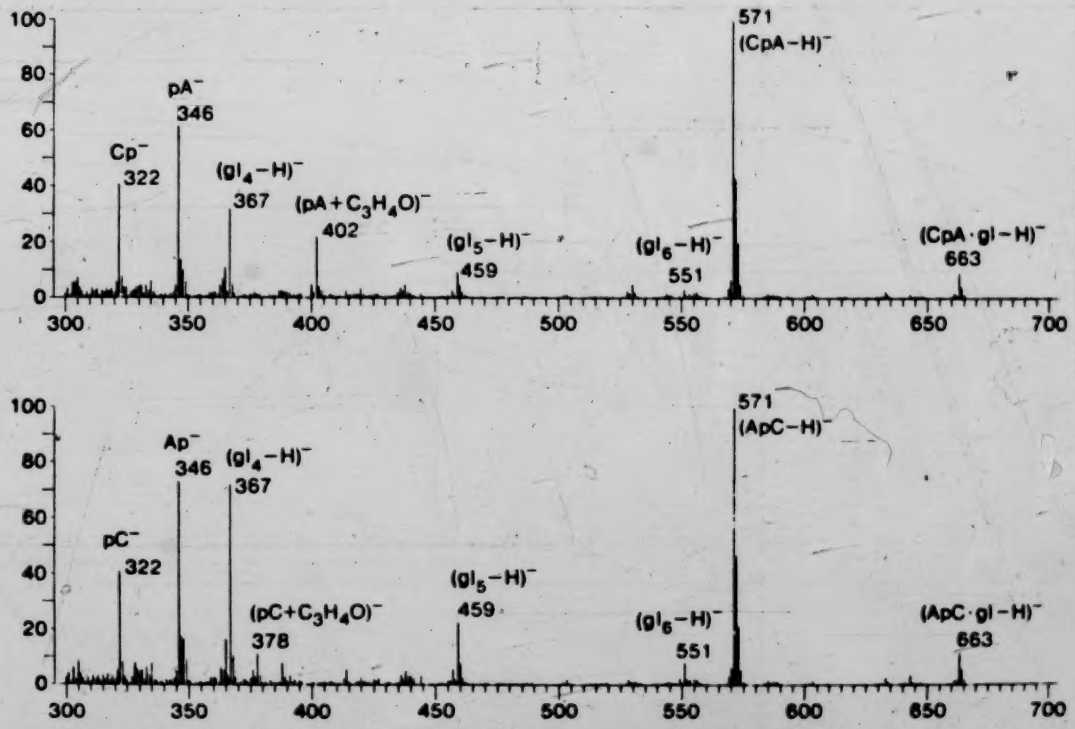


Figure 11. Negative ion FAB mass spectra of ApC and CpA.

sequence. The representative spectrum of d(TpTpC) is shown in Figure 12. The tetranucleotide d(TpTpTpTp) and the

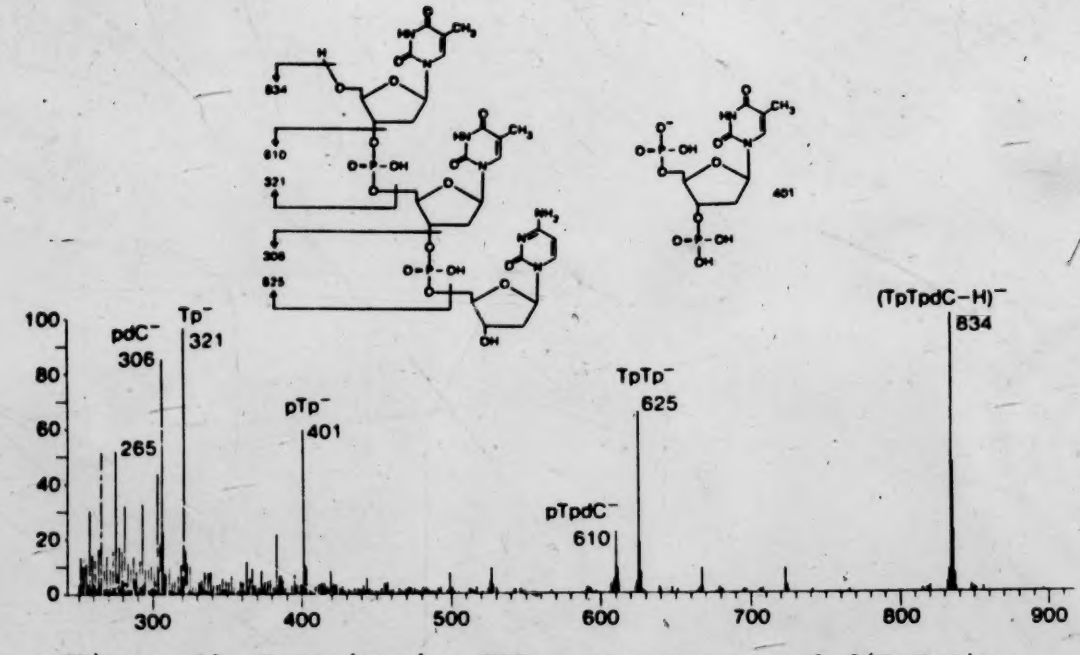


Figure 12. Negative ion FAB mass spectrum of d(TpTpC).

pentanucleotide d(TpTpTpTpTp) also give the expected negative ion FAB spectra. However, peaks corresponding to fission of the 5'-end sugar residue are very weak or absent in these spectra, and there is no obvious way to obtain directional information. Grotjahn et al[328] report in their study of one deca- and two octa-deoxyribonucleotides that the peaks corresponding to fragments with a terminal 5'-phosphate group were more intense than those with a terminal 3'-phosphate group. This might arise from the expected preferred cleavage of a phosphate anion from the secondary 3'-carbon rather than the primary 5'-carbon of a deoxyribose moiety. However, this was the case for only three of the trinucleotides we studied, namely, ApGpU, ApUpU

and ApApU. For all others, the terminal 3'-phosphate fragments gave peaks as or more intense than those bearing a terminal 5'-phosphate group. Panico et al[325] also found no correlation between peak intensities and position of terminal phosphate groups in their study of deoxyribotetranucleotides. Thus, no direct way exists of determining the direction of a small oligonucleotide sequence by negative ion FAB MS. Selective end modification may be necessary to achieve this.

The positive ion FAB mass spectra of oligonucleotides are not as useful as the negative ion spectra. Extensive cationization by even traces of sodium ions occurs and meaningful peaks are often lost in the matrix-derived background. This is illustrated for d(TpA) in Figure 13. The fragment peaks at 321 ($d(Tp)^-$) and 330 ($d(pA)^-$) are quite intense in the negative ion spectrum while the expected corresponding peaks at 323 and 332 are not seen above background level in the positive ion spectrum. Since the sequence fragments are more likely to have a negative charge, they would probably not be visible in the positive ion spectra in any case. Also, cationization by sodium severely complicates the molecular ion region in the positive ion mode. This is illustrated further by the positive ion spectra of ApApApApA and d(TpTpTpTpTpTp) (Figure 14). Little if any MH^+ is observed while sodium-containing ions are prominent. A previously-reported[288] positive ion FAB mass spectrum of d(TpTpTpTpTpTp) showed

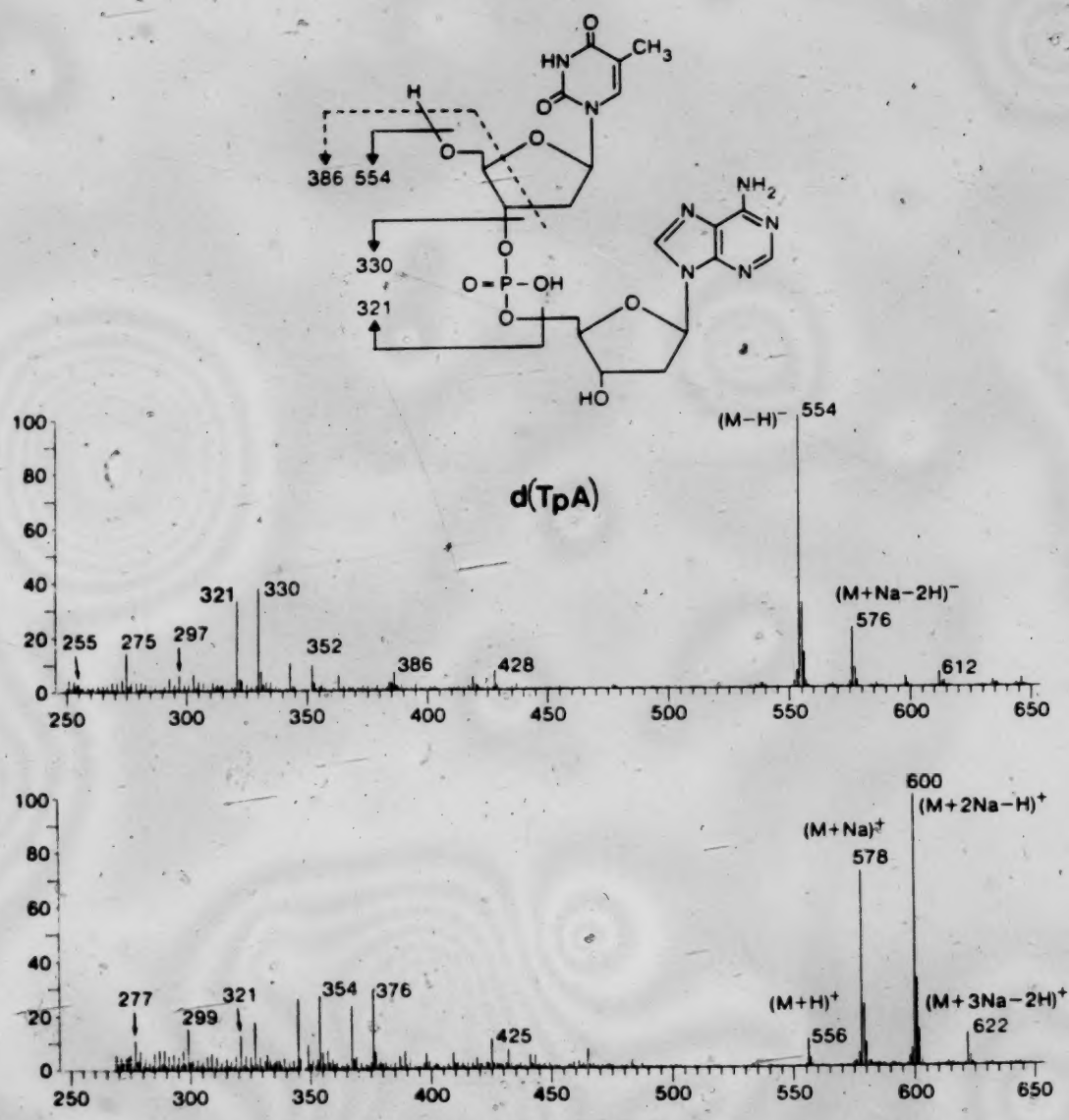


Figure 13. FAB mass spectra of d(TpA) in negative (above) and positive (below) ion modes.

less, but still extensive, sodium cationization. Aubagnac et al[326] also observed much sodium cationization and a high level of background noise in their positive ion FAB mass spectra of arabinose nucleotides.

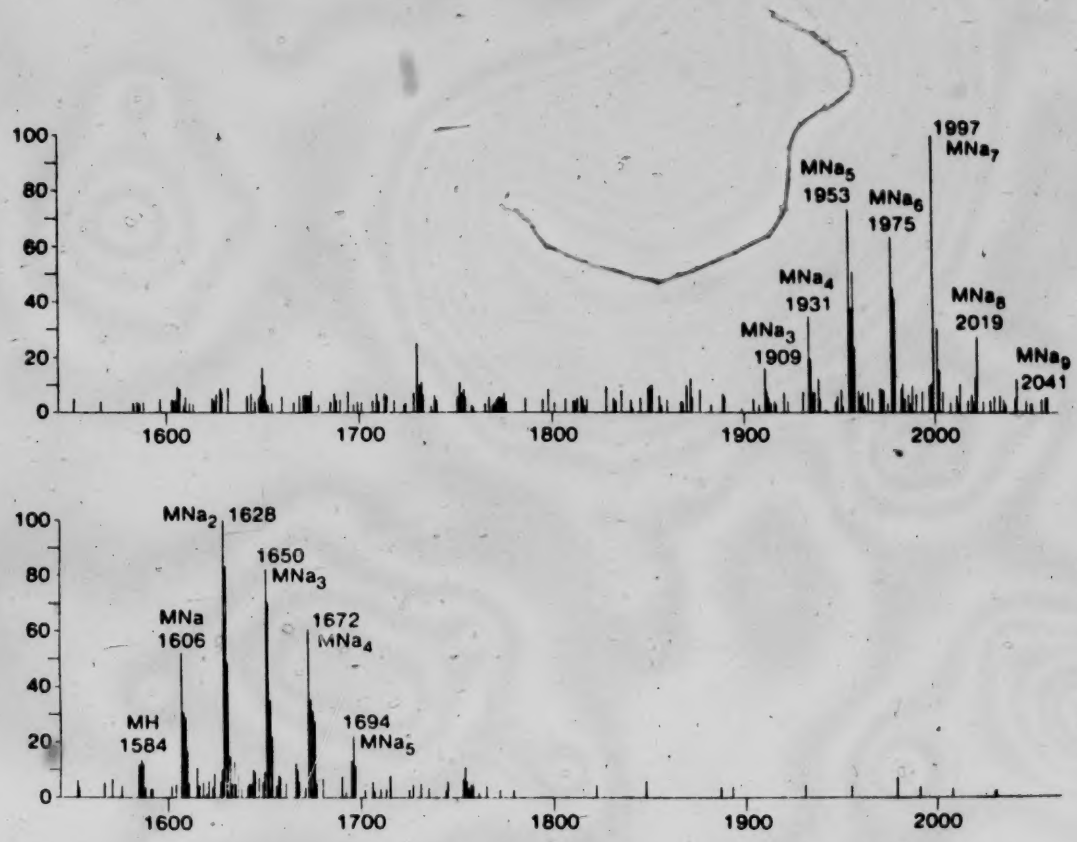
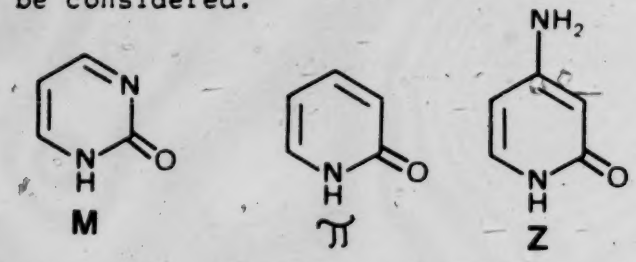


Figure 14. Positive ion mass spectra of d(TpTpTpTpTpTp) (above) and ApApApApA (below).

Several fully protected deoxyribonucleotides[337] were studied by FAB MS and in all cases, little if any quasi-molecular ion ($M-H^-$) was observed. However, loss of the cyanoethyl moiety occurred readily to give a prominent peak at 54 daltons lower mass. Ulrich et al[331] observed similar behaviour in their negative ion FAB mass spectra of fully protected dinucleotides. Loss of the p-chlorophenyl and dimethoxytrityl groups are also observed, as well as cleavage of the base-sugar and phosphate-sugar bonds. The fully protected nucleotide molecule does not have a proton

which can be easily lost to leave a stable negatively-charged fragment. As a result, loss of one of the protecting groups is necessary to obtain such a fragment. Fission of the sugar residue, as was seen in the case of the free dinucleotides occurs and loss of the base protecting group is observed in some cases. Grotjahn et al[332] observed similar fragmentations. The representative spectrum of fully protected thymidine is shown in Figure 15. The extension of this technique to unnatural nucleosides and nucleotides could now be considered.



The nucleosides containing the 2(1H)-pyrimidone (M), 2(1H)-pyridone (π) and 4-amino-2(1H)-pyridone (Z) bases[338-340] gave quasimolecular ion ($M-H^-$) peaks and showed fragmentation of the base-sugar bond. This is shown in the spectrum of dπ (Figure 16). This was also observed by Crow et al[323] in their FAB mass spectra of natural nucleosides. In the case of dM, a high matrix-derived background made interpretation of the spectrum difficult. Many peaks at higher mass than the quasimolecular ion were observed, due to clustering of the quasimolecular ion with molecules of glycerol or of itself.

The 5'-mono- or triphosphates of these nucleosides were also studied. For dπ-5'-MP (Figure 17), a significant

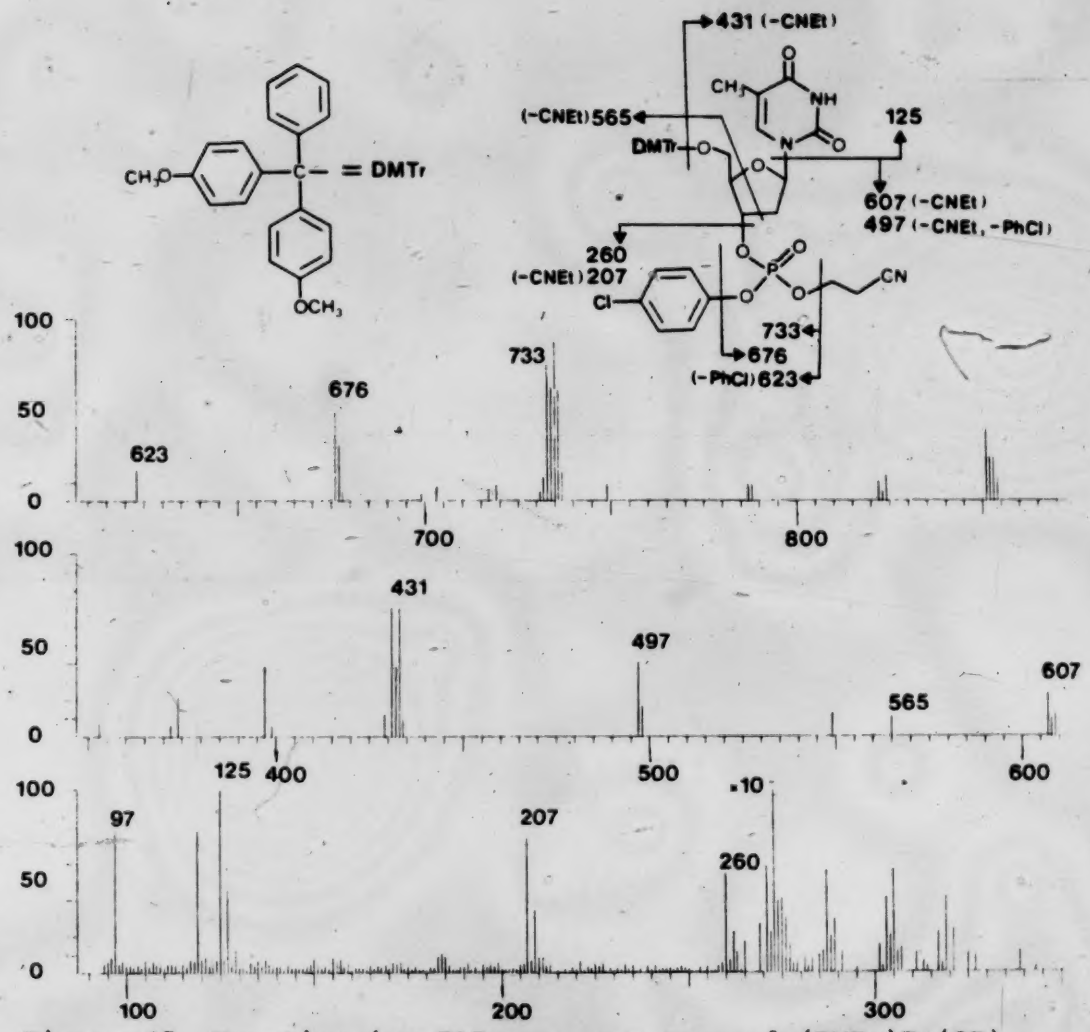


Figure 15. Negative ion FAB mass spectrum of (DMTr)Tφ(CE).

quasimolecular ion peak at 290 daltons was observed, as well as fragment peaks at 195 ($(M-HB+1)^-$) and 97 ($H_2PO_4^-$). A high degree of clustering of the quasimolecular ion with glycerol, H_3PO_4, the 195 dalton fragment and itself occurred. The highest peak observed in the spectrum was $(M_3-H)^-$ at 872 daltons, but this peak was of quite low intensity. For dZ-5'-TP, however, little fragmentation was

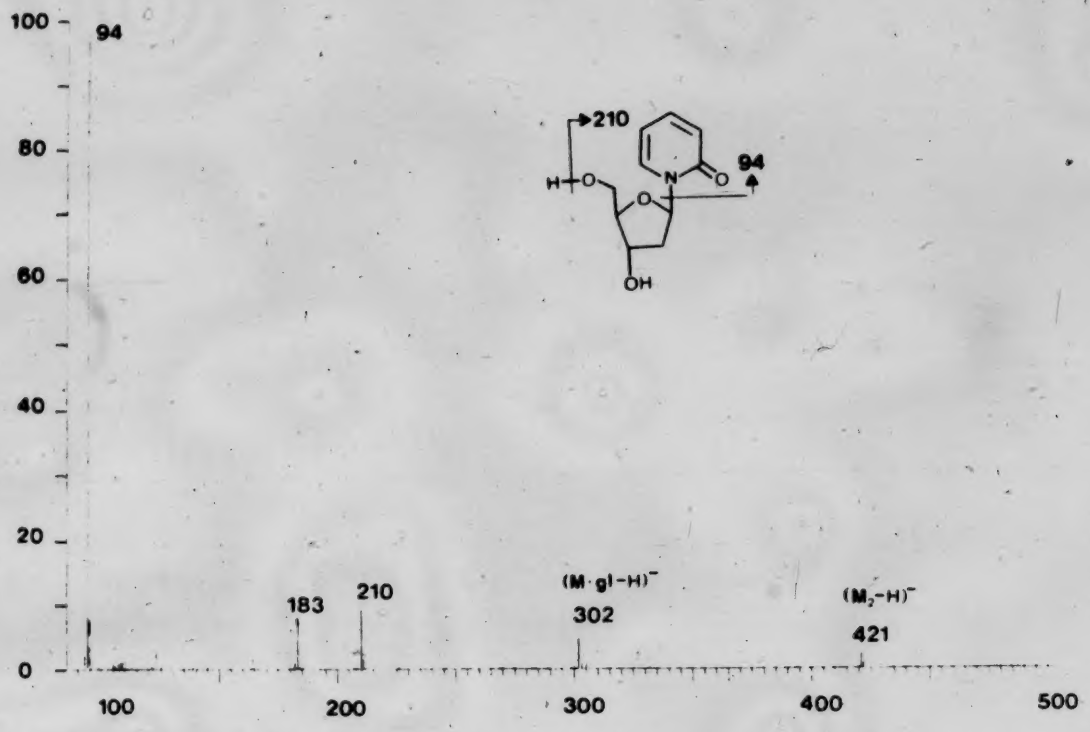


Figure 16. Negative ion FAB mass spectrum of dπ.

observed above the high background. It is possible that low solubility in the glycerol matrix caused a low intensity spectrum. The sodium-bound quasimolecular ion at 487 daltons is visible as well as glycerol-solvated quasimolecular ions at 579 and 671 daltons.

Dinucleotides containing unnatural bases appear to behave much like natural dinucleotides. For d(MpT), the quasimolecular ion at 515 daltons ($(M-H)^-$) is observed as well as the expected fragments at 291 (Mp^-), 321 (pT^-) and 377 ($(pT + C_3H_4O)^-$). The 5'-protected monomethoxytrityl d(MpC) was also studied (Figure 18). The expected fragmentations were seen, but it is interesting that little of the expected facile cleavage of the monomethoxytrityl

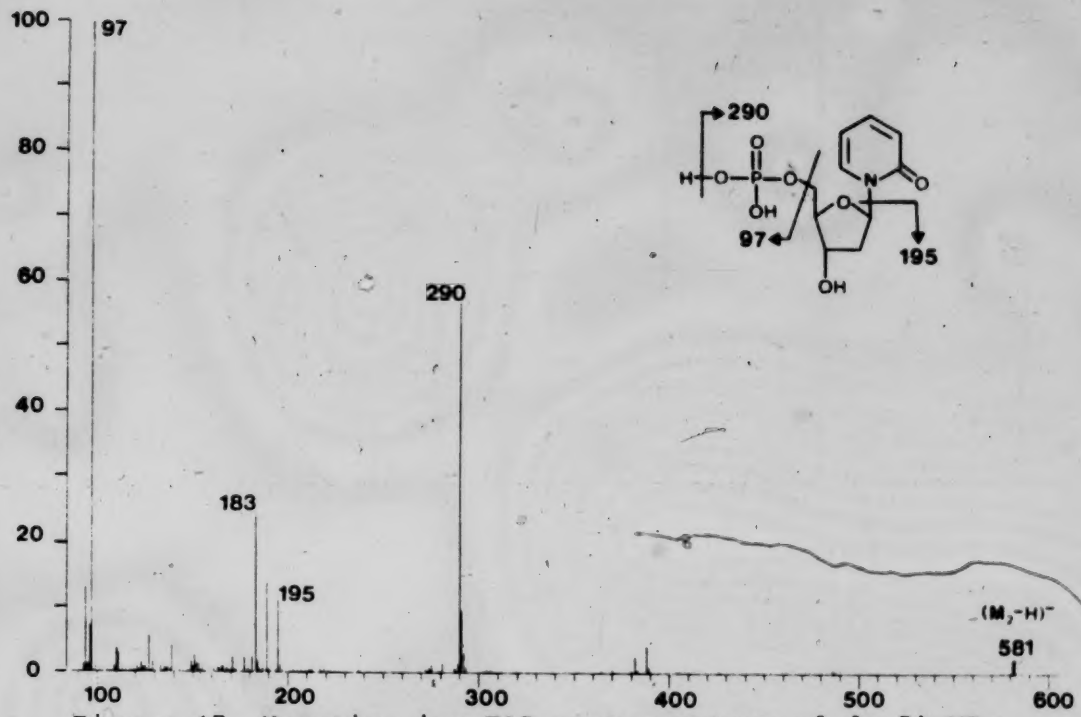


Figure 17. Negative ion FAB mass spectrum of dπ-5'-MP.

group was observed. While this cleavage was observed by Grotjahn et al[332], Ulrich et al[331] did not see any loss of this group in their study.

Nucleosides and nucleotides containing unnatural bases thus appear to behave quite similarly to the natural compounds and lend themselves well to study by fast atom bombardment mass spectrometry.

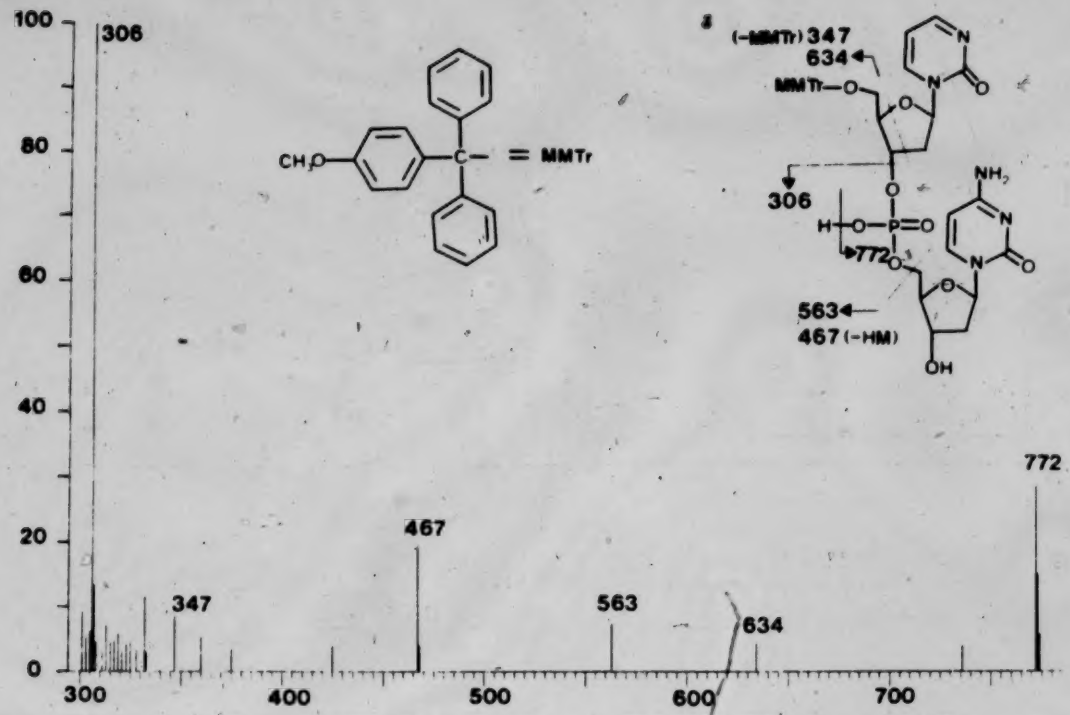


Figure 18. Negative ion mass spectrum of (MMTr)d(MpT).

Experimental

General

All melting points were determined on a Fisher-Johns capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR and mass spectra (MS) were recorded on A.E.I. MS-50 (high resolution) or MS-12 (low resolution) mass spectrometers at an ionizing voltage of 70 eV. Proton NMR spectra were recorded on Bruker WP-80 (80 MHz), Varian HA-100 (100 MHz), Bruker WH-200 (200 MHz) and Bruker (WH-400) (400 MHz) instruments in the specified deuterated solvent with tetramethylsilane (TMS) or deuterated sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) as internal standards. ^{19}F NMR were recorded on Bruker WP-80 (75.3 MHz) or Bruker HFX-90 (84.7 MHz) spectrometers with $CFCl_3$ as an internal or external standard. ^{13}C NMR were recorded on a Bruker WH-400 (100.6 MHz) spectrometer with TMS as an internal standard. Optical rotation measurements were recorded on a Perkin-Elmer 141 polarimeter using a 1 cm cell.

Silica gel for column chromatography was Merck type 60, 70-230 mesh. Flash chromatography was carried out by the method of Still *et al*[230], using Merck type 60 silica gel, 230-400 mesh. Commercial thin-layer chromatography (TLC) plates were silica, Merck 60F-254. Plates for preparative TLC were 20 x 20 x 0.2 cm and were heated at 100°C for 1-2 h before use. Silica gel for preparative TLC was Merck 60 PF254 or Merck 60 GF254. Spots were detected by absorption

of UV light or staining with iodine, ninhydrin (amino acids), bromocresol green spray (acids) or sulfuric acid spray with charring. TLC of amino acids was done on ion exchange sheets (Fixion 50X8, Na^+ form) in 0.4 M sodium citrate buffer.[267] Ion exchange chromatography employed Bio-Rad AG 50W-X8 cation exchange resin (50-100 mesh) or AG 1-X8 anion exchange resin (50-100 mesh).

Reagent grade solvents and reagents were used directly except for the following: ethyl acetate and Skellysolve B (hexanes) were distilled; tetrahydrofuran (THF) was distilled from sodium-benzophenone under argon atmosphere; ethanol and methanol were distilled from magnesium under argon atmosphere; diisopropylamine was distilled from calcium hydride under argon atmosphere.

Reactions done at 4°C were cooled in ice-water; reactions done at -78°C were cooled in dry ice-acetone. Unless otherwise noted, stirring refers to the use of a Teflon coated magnetic bar.

Enzymatic Studies

meso-Diaminopimelate decarboxylase was isolated from Bacillus sphaericus IFO 3525 by Dr. M.M. Palcic, using the method described by Asada et al[57], and from wheat germ (Triticum vulgaris, Sigma) by the method of Mazelis and Creveling.[37] The specific activities of these preparations were 16 units/mg and 0.017 units/mg respectively. Assays

were performed by measuring $^{14}CO_2$ evolution from [1,7-^{14}C]diaminopimelic acid. The assay mixtures (in scintillation vials) contained 50 mM potassium phosphate buffer, pH 6.8, 1.2 mM diaminopimelic acid, 0.25 μCi of [1,7-^{14}C]diaminopimelic acid, 50 μM pyridoxal phosphate, 1.2 mM ethylenediaminetetraacetic acid (EDTA), 3.5 mM dithioerythritol (DTE) and enzyme in a final volume of 0.86 mL. The caps of the vials contained a 1.5 x 1.5 cm piece of filter paper impregnated with 20 μL of 1 M hyamine hydroxide as a $^{14}CO_2$ trapping agent. The reaction was initiated by addition of enzyme, and the assay mixture was incubated at 25°C with shaking for 20 minutes. Reaction was terminated by addition of 0.2 mL of 10% trichloroacetic acid and the vials were shaken for an additional 60 min to assure that $^{14}CO_2$ evolution was complete. The filter paper was removed and counted in 10 mL of ACS scintillation cocktail on a Beckman LS100C scintillation counter. Assays were standardized with an excess of enzyme to completely release and trap CO_2 from substrate. One unit is defined as the amount of enzyme catalysing the release of 1 μmol of CO_2 per min under the above conditions. Protein concentrations were estimated using the Bio-Rad protein assay, based on Bradford's[341] method, using bovine serum albumin as a standard.

Assays of enzyme inhibition were accomplished as described above except that test material was included in the mixture. Assays for time-dependent inhibition were carried out by incubating test material with enzyme at 30°C

and withdrawing aliquots of this mixture at 0, 15 and 30 minutes incubation time for assay by the above procedure. Control experiments were performed simultaneously.

meso-Diaminopimelate-D-dehydrogenase was isolated from Bacillus sphaericus IFO 3525 by Dr. M.M. Palcic as described by Misono and Soda.[49] The specific activity of the preparation was 46 units/mg. Assays were performed by monitoring NADPH absorbance at 340 nm. The assay mixture (in a 1 mL cuvette) contained 0.2 M glycine-KCl buffer, pH 10.5, 2.5 mM diaminopimelic acid, 0.1 mM $NADP^+$ and enzyme in a final volume of 0.90 mL. The mixture was incubated at 25°C and reaction was initiated by addition of enzyme. Activity was measured by the rate of increase of absorbance at 340 nm, using a Beckmann DU-8 UV-visible spectrophotometer. One unit of enzyme catalyses the formation of 1 μmol of NADPH per min.

Assays of enzyme inhibition were carried out as described above, except that test material was included in the mixture. Assays for time dependent inhibition were carried out by incubating enzyme, test material and 0.8 mM $NADP^+$ and withdrawing aliquots of this mixture for assay as described above. Control experiments were performed simultaneously.

Fast Atom Bombardment Mass Spectra

Fast atom bombardment mass spectra were recorded on a Kratos/A.E.I. MS9 mass spectrometer with a mass range of 1400 daltons at an accelerating voltage of 6 kV, and on a

Kratos/A.E.I. MS50 mass spectrometer, with a mass range of 2300 daltons at an accelerating voltage of 6 kV. Both instruments are fitted with saddle-field fast atom guns, ion sources and copper sample probes as previously described.[326] Spectra were recorded at a scan rate of 10 s/decade using a Kratos DS55 on-line data system. Calibration of the data system for a mass range of 1300 daltons or less was carried out in the FAB mode for both positive and negative ions with perfluorotriheptyltriazine. Fomblin was used in the EI positive ion mode when mass ranges greater than 1300 daltons were required.

Commercially available free oligonucleotides (PL Biochemicals, Sigma, Amersham) were used without purification or after ion exchange chromatography on AG 50W-X8 (H^+ or NH_4^+ form, 50-100 mesh, Bio-Rad) or on DEAE-cellulose (Sigma) with water and 0.5 M ammonium formate (pH 6.5) or 0.5 M ammonia. Chromatographed samples were lyophilysed under high vacuum. Protected and unnatural nucleosides and nucleotides were prepared in the laboratory of Dr. C. Tamm, as previously described.[337-340] Sample loading was usually about 20 μg of nucleotide dissolved in 2 μL of glycerol or sulfolane/ glycerol. The saddle field discharge was ca. 1 mA at 5 kV applied voltage using xenon gas.

NMR Spectra of Lysine Biscamphanamides 102, 103 and 106

^1H, ^{13}C and ^1H-^{13}C shift correlation spectra were recorded on a Bruker WH-400 spectrometer. Typically, 20-50

mg of labelled material (**103** or **106**) was diluted with 10-20 mg of unlabelled **102** in 0.5 mL of C_6D_6 containing TMS and 15 drops of C_6F_6 in 5 mm tubes. Two-dimensional ^1H-^{13}C shift correlation experiments with ^2H-decoupling used (^{19}F lock on C_6F_6) used literature[250-253] pulse sequences with the following parameters: acquisition time, 0.098 s; f_1 (^1H) 1724 Hz; f_2 (^{13}C) 5208 Hz; relaxation delay 3 s between scans; 64-290 scans per increment; 1K FID's for 64 values of t_1; zero filling to 512 points in f_1 and 2K in f_2; and line broadening 6.0 and 5.0 for f_1 and f_2 respectively.

Methyl ornithinate dihydrochloride[163] (6)

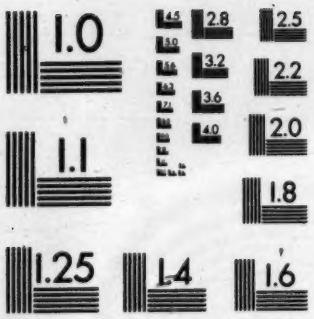
A suspension of L-ornithine hydrochloride (50 g, 0.30 mmol) in methanol (500 mL) was stirred at 4°C while thionyl chloride (53 g, 0.45 mol) was added dropwise. During the addition, the solid dissolved and the solution was stirred at room temperature for 78 h. Evaporation of the solvent <u>in vacuo</u> left 64.6 g (99%) of **6** as a white solid, m.p. 192-193°C (recrystallization from methanol/ether) (lit.[163] m.p. 192-194°C); IR ($CHCl_3$/MeOH cast) 2900, 1745 cm^{-1}; ^1H NMR (CD_3OD, 80 MHz) δ 4.12 (m, 1H, C*H*), 3.90 (s, 3H, COOC*H*$_3$), 3.05 (m, 2H, C*H*$_2$N), 2.03 (m, 4H, *CH*$_2$); MS (FAB) 407 (M_2Na^+ + glycerol), 329 (M_2H^+ + HCl), 293 (M_2H^+), 261 (MNa^+ + glycerol), 147 (MH^+), 132 ($MH^+ - CH_3$), 130 ($MH^+ - NH_3$).

Methyl <u>N</u>^{5}-(<u>p</u>-tolylsulfonyl)ornithinate hydrochloride[165,166] (7)

To a stirred, cooled (4°C) suspension of 6 (2.0 g, 9.1 mmol) in chloroform (100 mL) was added triethylamine (2.8 g, 27 mmol) and p-toluenesulfonyl chloride (1.7 g, 9.1 mmol). Stirring was continued at room temperature for 24 h and the solvent was evaporated *in vacuo*. The residue was partitioned between dichloromethane (2 x 50 mL) and water (50 mL) containing saturated aqueous potassium bicarbonate (5 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated under reduced pressure. The white solid residue was dissolved in ether (150 mL) and dichloromethane (80 mL) and hydrogen chloride gas was passed over the surface of the stirred, cooled (4°C) solution for 15 min. After concentration of the solution to *ca.* half its volume, a white solid precipitated and was collected by filtration. This was identified as 7 (1.99 g, 65%), m.p. 133-135°C (lit.[165] m.p. 135-136°C); IR ($CHCl_3$/MeOH cast) 1745, 1330, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 8.50 (br s, 3H, NH_3^+), 7.78 (d, J = 8 Hz, 2H, H_{ortho} to SO_2), 7.30 (d, J = 8 Hz, 2H, H_{ortho} to CH_3), 6.70 (m, 1H, NH), 4.35 (m, 1H, CH), 3.78 (s, 3H, $COOCH_3$), 2.95 (m, 2H, CH_2NH), 2.40 (s, 3H, $PhCH_3$), 2.18 (m, 2H, CH_2); MS (FAB) 601 (M_2H^+), 301 (MH^+), 224 (M - COOMe, NH_3), 155 (p-$MePhSO_2^-$)

Methyl N^2-benzoyl-N^5-(p-tolylsulfonyl)ornithinate (8)

Triethylamine (0.61 g, 6.0 mmol) and benzoyl chloride (0.42 g, 3.0 mmol) were added to a solution of 7 (1.0 g, 3.0 mmol) in chloroform (50 mL). The mixture was stirred at room



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temperature 64 h, and was washed with saturated aqueous potassium bicarbonate (30 mL). The aqueous phase was extracted with chloroform (50 mL) and the combined chloroform extracts were dried (Na_2SO_4) and concentrated *in vacuo* to leave 1.60 g (78% purity) of crude **8** as a white solid. Most of this was used without further purification, but a small sample was purified by flash chromatography[230] (50% dichloromethane/ethyl acetate) followed by recrystallization from ethyl acetate/hexane to give white needles, m.p. 134.5-135°C; IR ($CHCl_3$ cast) 1740, 1640, 1325, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.50 (m, 9H, *Ph*), 6.95 (d, J = 8 Hz, 1H, CON*H*), 5.35 (t, J = 6 Hz, 1H, SO_2NH), 4.78 (dt, J = 7 Hz, 8 Hz, 1H, C*H*), 3.73 (s, 3H, $COOCH_3$), 2.98 (dt, J = 6 Hz, 6 Hz, 1H, CH_2NH), 2.38 (s, 3H, $PhCH_3$), 2.1-2.4 (m, 4H, CH_2); exact mass 404.1383 (404.1406 calc. for $C_{20}H_{24}N_2O_5S$); Anal. Calc. for $C_{20}H_{24}N_2O_5S$: C, 59.39; H, 5.98; N, 6.93; S, 7.93. Found: C, 59.50; H, 5.98; N, 6.72; S, 7.91.

Methyl N^2-benzoyl-N^5-(p-tolylsulfonyl)-N^5-(p-nitrophenylsulfonyl)ornithinate (9)

Sodium hydride (50% dispersion in oil, 70 mg, 1.5 mmol) was washed with THF (2 x 5 mL) and resuspended in THF (5 mL) with stirring under argon atmosphere. A solution of **8** (78% purity, 0.50 g, 0.96 mmol) in THF (10 mL) and methanol (0.6 mmol, 1.5 μL) were added and stirring was continued 30 min. A solution of p-nitrobenzenesulfonyl chloride (0.28 g, 1.3

mmol) in THF (8 mL) was added and stirring was continued at room temperature 46 h. Saturated aqueous sodium chloride (20 mL) was added and the mixture was extracted with dichloromethane (60 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated in vacuo. Column chromatography (chloroform) of the residue gave 516 mg (91%) of **9** as an amorphous solid; IR ($CHCl_3$ cast) 1740, 1650, 1530, 1375, 1350, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.24 (d, J = 9 Hz, 2H, H_{ortho} to NO_2), 8.11 (d, J = 9 Hz, 2H, H_{ortho} to SO_2 (Ns)), 7.81 (d, J = 8 Hz, 2H, H_{ortho} to SO_2 (Ts)), 7.74 (d, J = 8 Hz, 2H, H_{ortho} to CH_3), 7.40 (m, 3H, *Ph*), 7.23 (m, 2H, *Ph*), 6.71 (d, J = 7.5 Hz, 1H, CON*H*), 4.77 (dt, J = 7.5 Hz, 5 Hz, 1H, C*H*COOMe), 3.76 (m, 5 H, $COOCH_3$, CH_2N), 2.40 (s, 3H, $PhCH_3$), 1.9 (m, 4H, CH_2); exact mass 589.1195 (589.1189 calc. for $C_{26}H_{27}N_3O_9S_2$). Anal. Calc. for $C_{26}H_{27}N_3O_9S_2$: C, 52.96; H, 4.62; N, 7.13; S, 10.87. Found: C, 53.34; H, 4.70; N, 6.98; S, 10.84.

2-Phenyl-1,3-dioxane[168] (11)

The procedure of Salmi[169] was followed. A mixture of benzaldehyde (31.8 g, 0.30 mol), 1,3-propanediol (25 g, 0.33 mol) and p-toluenesulfonic acid (0.57 g, 3.0 mmol) in benzene (500 mL) was heated at reflux for 6 h with azeotropic removal of water. The solvent was evaporated in vacuo to leave a pale yellow oil which was crystallized from petroleum ether to yield 39.6 g (80%) of **11** as large

colorless crystals, m.p. 44-46°C (lit.[168] m.p. 49-51°C); IR ($CHCl_3$ cast) 1110 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.40 (m, 5H, *Ph*), 5.45 (s, 1H, *CH*), 4.00 (m, 4H, OCH_2), 2.20 (m, 1H, H_{ax} of CH_2), 1.42 (m, 1H, H_{eq} of CH_2); exact mass 164.0828 (164.0837 calc. for $C_{10}H_{12}O_2$).

3-Benzyloxy-1-propanol[170] (12)

The procedure of Eliel *et al*[170] was used. A mixture of aluminum chloride (13.5 g, 0.101 mol) and dry ether (100 mL) was stirred under argon atmosphere at 4°C for 40 min. Lithium aluminum hydride (0.95 g, 25 mmol) was added as a suspension in ether (28 mL) and stirring was continued for 30 min. A solution of **11** (8.2 g, 50 mmol) in ether (100 mL) was added and stirring was continued at room temperature for 2 h. Aqueous 10% sulfuric acid (100 mL) was added dropwise, with cooling of the mixture to 4°C, and the phases were separated. The aqueous phase was washed with ether (3 x 50 mL) and the combined ether extracts were dried (K_2CO_3) and concentrated *in vacuo* to leave a pale yellow liquid. Distillation under reduced pressure gave 4.93 g (59%) of **12**, b.p. 70-74°C (0.05 mm Hg) (lit.[170] b.p. 110°C (0.5 mm Hg)); IR ($CHCl_3$ cast) 3400, 1100, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.23 (s, 5H, *Ph*), 4.42 (s, 2H, $PhCH_2$), 3.63 (t, J = 6 Hz, 2H, CH_2OH), 3.53 (t, J = 6 Hz, 2H, CH_2O), 3.28 (s, 1H, *OH*), 1.78 (tt, J = 6Hz , J = 6Hz, 2H, CH_2); exact mass 166.0991 (166.0994 calc. for $C_{10}H_{14}O_2$).

3-Benzyloxy-1-propyl p-toluenesulfonate[171] (13)

The procedure of Butler *et al*[171] was modified. Pyridine (2.86 g, 36.0 mmol) and p-toluenesulfonyl chloride (2.52 g, 13.2 mmol) were added to a stirred, cooled (4°C) solution of 12 (2.0 g, 12 mmol) in dichloromethane (100 mL) and stirring was continued 68 h at room temperature. The mixture was washed with water (50 mL) and 10% aqueous potassium bicarbonate (40 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residual oily solid was suspended in ether (70 mL) and the remaining white solid was filtered. The filtrate was concentrated *in vacuo* to leave a clear oil. Column chromatography (gradient elution with methanol/ chloroform) gave 13 (2.49 g, 65%) as an oil which crystallized on standing at room temperature, m.p. 34-35°C (lit.[171] m.p. 37°C) and 86 mg (4%) of a clear oil which was identified as 1-benzyloxy-3-chloropropane[172] (14). Alternatively, purification of 13 could be achieved by flash chromatography[230] (27% ethyl acetate/hexane). For 13; IR ($CHCl_3$ cast) 1360, 1190, 1180 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.76 (d, J = 8 Hz, 2H, H_{ortho} to SO_2), 7.30 (d, J = 8 Hz, 2H, H_{ortho} to CH_3), 7.25 (s, 5H, *Ph*), 4.40 (s, 2H, $PhCH_2$), 4.17 (t, J = 6 Hz, 2H, CH_2OTs), 3.50 (t, J = 6 Hz, 2H, OCH_2), 2.40 (s, 3H, $PhCH_3$), 1.93 (tt, J = 6 Hz, J = 6 Hz, 2H, CH_2); exact mass 320.1085 (320.1082 calc. for $C_{17}H_{20}O_4S$). For 14; IR (CH_2Cl_2 cast) 1180 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.29 (s, 5H, *Ph*), 4.50 (s, 2H, $PhCH_2$), 3.65 (t, J = 6 Hz, 2H, CH_2Cl), 3.59 (t, J = 6 Hz, 2H, CH_2O),

2.04 (tt, J = 6 Hz, J = 6 Hz, 2H, CH_2); exact mass 186.0619 and 184.0647 (186.0625 and 184.0655 calc. for $C_{10}H_{13}ClO$).

1-Benzyloxy-3-iodopropane[173] **(15)**

The procedure followed was that of Place *et al.*[174] Magnesium metal (1.5 g, 60 mmol) and dry ether (50 mL) were stirred under argon atmosphere at room temperature while iodine (12.7 g, 50.0 mmol) was added in portions. As addition of iodine neared completion, the mixture began to boil and continued to do so for 1 h, at which point the brown color of iodine had disappeared. The greenish solution was diluted to 90 mL and a portion (20 mL, 11.2 mmol of MgI_2) was transferred by syringe to another flask under argon atmosphere. A solution of **13** (1.6 g, 5.0 mmol) in ether (7 mL) was added and a yellow solid formed immediately. The mixture was stirred at room temperature 35 min and ice water (10 mL) was added. The aqueous phase was separated and washed with ether (3 x 25 mL) and the yellow ether extracts were combined and washed with 5% aqueous sodium thiosulfate (10 mL) to decolorise. Drying (Na_2SO_4) of the ether extract and evaporation of the solvent *in vacuo* left 1.20 g (87%) of **15**; IR ($CHCl_3$ cast) 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.28 (s, 5H, *Ph*), 4.43 (s, 2H, $PhCH_2$), 3.50 (t, J = 6 Hz, 2H, CH_2O), 3.26 (t, J = 6 Hz, 2H, CH_2I), 2.05 (tt, J = 6 Hz, 6 Hz, 2H, CH_2); exact mass 276.0014 (276.0011 calc. for $C_{10}H_{13}IO$).

Diethyl (acetamido)(3-benzyloxy-1-propyl)malonate (17)

The procedure of Dean and Rapoport[175] was used with slight modification. Sodium hydride (50% in oil, 0.48 g, 10 mmol) was washed with THF (2 x 5 mL) under argon atmosphere, then was suspended with stirring in DMF (20 mL). Diethyl acetamidomalonate (2.17 g, 10.0 mmol) was added and the mixture was stirred at room temperature for 2.5 h. Stirring was discontinued and the supernatant solution was transferred by syringe into a second flask under argon atmosphere containing 15 (2.51 g, 9.09 mmol). The remaining solid was washed with DMF (5 mL) and the washings were transferred to the second flask. This mixture was stirred at room temperature for 25 h, and was partitioned between water (100 mL) and dichloromethane (2 x 100 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a dark orange oil. Column chromatography (chloroform; 2% methanol/ chloroform) gave 2.65 g (80%) of 17 as a clear oil; IR ($CHCl_3$ cast) 1740, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.28 (s, 5H, *Ph*), 6.80 (br s, 1H, *NH*), 4.46 (s, 2H, Ph*CH*$_2$), 4.23 (q, J = 7 Hz, 4H, CH_3CH_2), 3.45 (t, J = 6 Hz, 2H, *CH*$_2$O), 2.42 (m, 2H, *CH*$_2$C(COOEt)$_2$), 2.00 (s, 3H, CH_3CO), 1.7-1.4 (m, 2H, *CH*$_2$), 1.22 (t, J = 7 Hz, CH_3CH_2); exact mass 365.1842 (365.1838 calc. for $C_{19}H_{27}NO_6$); Anal. Calc. for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.40; H, 7.08; N, 3.57.

Diethyl (acetamido)(3-hydroxy-1-propyl)malonate[176] (18)

A solution of 17 (1.0 g, 2.7 mmol) in ethyl acetate (50 mL) was added to 5% palladium on charcoal (106 mg) which was premoistened with ethyl acetate. The mixture was stirred under hydrogen (atmospheric pressure) for 20 h and the catalyst was removed by filtration through a Celite pad. Evaporation of the solvent in vacuo gave a pale yellow oil which was purified by flash chromatography[230] (55% acetone/hexane) to leave 18 as an oil which crystallized at room temperature (661 mg, 89%), m.p. 77-78.5°C (lit.[176] m.p. 81-82°C); IR ($CHCl_3$ cast) 3380 (br), 1740, 1660 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.07 (s, 1H, NH), 4.22 (q, J = 7 Hz, 4H, CH_3CH_2), 3.55 (br t, J = 6 Hz, 2H, CH_2OH), 3.22 (br s, 1H, OH), 2.35 (m, 2H, $CH_2C(COOEt)_2$), 2.02 (s, 3H, CH_3CO), 1.61-1.15 (m incorporating t at 1.25, J = 7 Hz, 8H, CH_2, CH_2CH_3); exact mass 202.1087 (202.1079 calc for $C_9H_{16}NO_4$ (M - COOEt)).

Diethyl (acetamido)(3-[p-tolylsulfonyloxy]-1-propyl)malonate (19)

A solution of 18 (636 mg, 2.31 mmol) in dichloromethane (25 mL) was stirred at 4°C while pyridine (0.54 g, 6.9 mmol) and p-toluenesulfonyl chloride (0.48 g, 2.5 mmol) were added. Stirring was continued at room temperature for 140 h, and the mixture was washed with water (15 mL), dried (Na_2SO_4) and concentrated in vacuo to leave a yellow oil which crystallized at room temperature. Purification by flash chromatography[230] (10% ethyl acetate/ dichloromethane)

yielded 610 mg (62%) of **19** as a white solid, m.p. 102-103.5°C; IR ($CHCl_3$ cast) 1740, 1680, 1360, 1180 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.73 (d, J = 8 Hz, 2H, H_{ortho} to SO_2), 7.31 (d, J = 8 Hz, 2H, H_{ortho} to CH_3), 6.76 (s, 1H, NH), 4.22 (q, J = 7 Hz, 4H, CH_3CH_2), 3.98 (t, J = 6 Hz, 2H, CH_2O), 2.44 (s, 3H, CH_3Ph), 2.5-2.2 (m, 2H, $CH_2C(COOEt)_2$), 2.00 (s, 3H, CH_3CO), 1.8-1.4 (m, 2H, CH_2), 1.24 (t, J = 7 Hz, 6H, CH_2CH_3); exact mass 429.1460 (429.1457 calc. for $C_{19}H_{27}NO_8S$); Anal. Calc. for $C_{19}H_{27}NO_8S$: C, 53.14; H, 6.34; N, 3.26; S, 7.46. Found: C, 53.18; H, 6.39; N, 3.42; S, 7.23.

Diethyl (acetamido)(3-iodo-1-propyl)malonate (20)

The procedure of Place et al[174] was used. Magnesium metal (1.5 g, 60 mmol) was suspended in dry ether (100 mL) and stirred while iodine (12.7 g, 50.0 mmol) was added in small portions. When the color of the iodine had disappeared (35 min), the solution (2.0 mL, 1.0 mmol of MgI_2) was transferred to another flask under argon atmosphere, and a solution of **19** (200 mg, 0.52 mmol) in THF (5 mL) was added. The mixture was stirred 4 h at room temperature, and ice water (5 mL) was added. The phases were separated and the aqueous phase was washed with ether (3 x 5 mL). The combined ether extracts were dried (Na_2SO_4) and concentrated in vacuo to leave 149 mg (78%) of chromatographically pure **20** as a viscous oil which solidified at room temperature. A small analytical sample was recystallized from ether/ light

petroleum to give needles, m.p. 70-70.5°C; IR ($CHCl_3$ cast) 1740, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 6.84 (s, 1H, NH), 4.47 (q, J = 7 Hz, 4H, CH_3CH_2), 3.13 (t, J = 7 Hz, 2H, CH_2I), 2.6-2.3 (m, 2H, $CH_2C(COOEt)_2$), 2.03 (s,3H, CH_3CO), 1.9-1.5 (m, 2H, CH_2), 1.26 (t, J = 7 Hz, CH_2CH_3); exact mass 312.0088 (312.0097 calc. for $C_9H_{15}INO_3$ (M - COOEt)); Anal. Calc. for $C_{12}H_{20}INO_5$: C, 37.42; H, 5.23; N, 3.64. Found: C, 37.60; H, 5.26; N, 3.70.

Ethyl N-benzylideneglycinate[177] (21)

The procedure used was that of Stork et al.[177] A mixture of ethyl glycinate hydrochloride (10.1 g, 72.4 mmol), benzaldehyde (7.70 g, 72.4 mmol), triethylamine (14.5 g, 143 mmol) and magnesium sulfate (6 g) in dichloromethane (150 mL) was stirred at room temperature 22 h. Moisture was excluded from the mixture by means of a drying tube containing Drierite (calcium sulfate). The solvent was removed by evaporation in vacuo and the residue was partitioned between ether (100 mL) and water (100 mL). The organic phase was washed with saturated aqueous sodium chloride (50 mL), dried (Na_2SO_4) and concentrated in vacuo to leave 11.3 g (82%) of 21 as a pale yellow liquid; IR ($CHCl_3$ cast) 1745, 1647, 1187 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.22 (s, 1H, PhCH), 7.65 (m, 2H, Ph), 7.26 (m, 3H, Ph), 4.24 (s, 2H, CH_2), 4.07 (q, J = 7 Hz, 2H, CH_2CH_3), 1.13 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 191.0941 (191.0946 calc. for $C_{11}H_{13}NO_2$).

Ethyl 2-(benzylideneamino)-5-benzyloxypentanoate (23)

The procedure used was that of Stork *et al.*[177] A mixture of diisopropylamine (0.53 g, 5.2 mmol), HMPA (9.7 mL) and THF (50 mL) was stirred and cooled to -78°C under argon atmosphere. Butyllithium (1.22 M in hexane, 4.3 mL, 5.2 mmol) was added slowly and stirring was continued at -78°C for 15 min. A solution of **21** (1.0 g, 5.2 mmol) in THF (8 mL) was added, followed by a solution of **15** (2.0 g, 5.2 mmol) in THF (8 mL). The mixture was allowed to warm to room temperature and stirring was continued for 4 h. The solution was partitioned between ether (200 mL) and ice-cold 10% aqueous ammonium chloride (100 mL). The ether extract was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was partitioned between dichloromethane (100 mL) and water (100 mL) and the dichloromethane extract was dried (Na_2SO_4) and concentrated *in vacuo* to leave crude **23** as an unstable orange oil (1.97 g). This was not purified further, but was carried on in further reactions; IR ($CHCl_3$ cast) 1735, 1640, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.22 (s, 1H, PhC*H*=N), 7.73 (m, 2H, *Ph*), 7.30 (m, 8H, *Ph*), 4.46 (s, 2H, PhCH_2), 4.18 (q, J = 7 Hz, 2H, CH_2CH$_3$), 3.95 (m, 1H, C*H*COOEt), 3.48 (br t, J = 6 Hz, 2H, CH_2O), 2.3-1.4 (m, 4H, CH_2), 1.24 (t, J = 7 Hz, 3H, CH$_2$CH_3); exact mass 339.1834 (339.1834 calc for $C_{21}H_{25}NO_3$).

Ethyl 2-(benzamido)-5-benzyloxypentanoate (24)

Crude **23** (250 mg, ca. 66 mmol) was loaded onto a column of silica gel (70-230 mesh) and eluted with hexane, then ether to hydrolyse the imine. The ether eluate was concentrated *in vacuo* to leave 110 mg of a pale brown oil. To a solution of this oil (90 mg) in dichloromethane (5 mL) was added triethylamine (36 mg, 0.36 mmol) and benzoyl chloride (50 mg, 0.36 mmol) and the mixture was stirred at room temperature for 19 h. The solution was washed with saturated aqueous potassium bicarbonate (5 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography ($CHCl_3$) to leave 79 mg (34% from **21**) of **24** as a colorless oil; IR ($CHCl_3$ cast) 1735, 1645, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.2-6.8 (m, 11H, *Ph*, *NH*), 4.83 (m, 1H, *CH*COOEt), 4.48 (s, 2H, PhCH_2), 4.22 (q, J = 7 Hz, 2H, CH_2CH_3), 3.51 (m, 2H, CH_2O), 2.3-1.5 (m, 4H, CH_2), 1.28 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 355.1780 (355.1784 calc. for $C_{21}H_{25}NO_4$).

Ethyl 2-benzylamino-5-benzyloxy-2-(difluoromethyl)pentanoate (25)

For the initial part of this reaction, the procedure of Bey *et al*[161] was used with some modification. A mixture of diisopropylamine (0.60 g, 5.9 mmol) and THF (50 mL) was stirred and cooled to -78°C under argon atmosphere while butyllithium (1.6 M in hexane, 3.7 mL, 5.9 mmol) was added slowly. Stirring was continued at -78°C for 30 min, and a solution of crude **23** (2.0 g, ca. 5.9 mmol) in THF (8 mL) was

added. The dark orange solution was warmed to 40°C and the argon inlet was closed while chlorodifluoromethane gas was passed rapidly into the mixture until an attached balloon began to expand. The color of the mixture lightened during the addition. Stirring was continued at 40-50°C for 65 min, and the mixture was partitioned between ether (200 mL) and saturated aqueous sodium chloride (100 mL). The ether extract was dried (Na_2SO_4) and concentrated in vacuo to leave an oil (1.86 g). A portion of this (1.5 g) was dissolved in methanol (10 mL) and sodium cyanoborohydride (0.32 g, 5.1 mmol) and acetic acid (0.23 g, 3.9 mmol) were added. The mixture was stirred at room temperature for 46 h, and was then partitioned between 10% aqueous potassium bicarbonate (50 mL) and dichloromethane (2 x 50 mL). The combined organic extracts were washed with water (50 mL), dried (Na_2SO_4) and concentrated in vacuo. The residue was fractionated by flash chromatography[230] (20% ethyl acetate/hexane) to afford 470 mg (30% from 21) of 25 as a colorless oil, and 126 mg (7% from 21) of a colorless oil identified as ethyl 2-benzylamino-5-benzyloxy-2-(3-benzyloxy-1-propyl)pentanoate (26). For 25; IR ($CHCl_3$ cast) 1735, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.25 (s, 10H, *Ph*), 5.95 (t, J = 55 Hz, 1H, CHF_2), 4.45 (s, 2H, $PhCH_2O$), 4,23 (q, J = 7 Hz, 2H, CH_2CH_3), 3.82 (s, 2H, $PhCH_2N$), 3.45 (t, J = 6 Hz, 2H, CH_2O), 2.15-1.50 (m, 5H, CH_2, *NH*), 1.27 (t, J = 7 Hz, 3H, CH_2CH_3); ^{19}F NMR ($CDCl_3$, 75.3 MHz) δ -128.0 (ABX system, J_{HF} = 55 Hz, J_{FF} = 284 Hz); exact mass 391.1934 (391.1959

calc. for $C_{22}H_{27}F_2NO_3$); Anal. Calc. for $C_{22}H_{27}F_2NO_3$: C, 67.50; H, 6.95; N, 3.58. Found: C, 67.78; H, 7.08; N, 3.35. For **26**; IR ($CHCl_3$ cast) 1725, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.25 (s, 15H, *Ph*), 4.46 (s, 4H, $PhCH_2O$), 4.16 (q, J = 7 Hz, 2H, CH_2CH_3), 3.57 (s, 2H, $PhCH_2N$), 3.44 (br t, J = 6 Hz, 4H, CH_2O), 2.0-1.4 (m, 9H, CH_2, *NH*), 1.26 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 488.2814 (488.2801 calc. for $C_{31}H_{38}NO_4$ (M - H)); Anal. Calc. for $C_{31}H_{39}NO_4$: C, 76.04; H, 8.03; N, 2.86. Found: C, 76.01; H, 7.93; N, 2.87.

In a separate preparation, an oil which was identified as butyl 2-benzylamino-5-benzyloxy-2-(difluoromethyl)-pentanoate (**27**) was isolated in 10% yield from **21**; IR ($CHCl_3$ cast), 1735, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.30 (s, 10H, *Ph*), 5.97 (t, J = 56 Hz, 1H, CHF_2), 4.47 (s, 2H, $PhCH_2O$), 4.20 (t, J = 6 Hz, 2H, $COOCH_2$), 3.83 (s, 2H, $PhCH_2N$), 3.47 (t, J = 6 Hz, CH_2O), 2.3-0.8 (m, 12H, CH_3, CH_2, *NH*); ^{19}F NMR ($CDCl_3$, 75.3 MHz) δ -127.9 (ABX system, J_{HF} = 56 Hz, J_{FF} = 282 Hz); exact mass 419.2273 (419.2272 calc. for $C_{24}H_{31}F_2NO_3$); Anal. Calc. for $C_{24}H_{31}F_2NO_3$: C, 68.71; H, 7.45; N, 3.34. Found: C, 68.96; H, 7.45; N, 3.15.

4,5-Diphenyl-1,3-dioxol-2-one[181] **(30)**

The procedure followed was that of Sheehan and Guziec.[181] To a stirred mixture of benzoin (0.50 mol, 106 g) in benzene (400 mL) cooled to 4°C was added phosgene (0.55 mol, 54 g, 38 mL condensed at -78°C) by distillation. When addition was complete, dimethylaniline (0.50 mol, 61 g) was

added slowly through a dropping funnel. The mixture was allowed to warm to room temperature and stirred 15 h, then was cooled in ice and filtered. The filtrate was heated at reflux 3 h, and washed with 0.5 M hydrochloric acid (250 mL) and water (250 mL). The organic extract was dried (Na_2SO_4) and evaporated in vacuo. The residual blue oil was again dissolved in benzene (250 mL) and washed with 0.5 M hydrochloric acid (2 x 150 mL) and saturated aqueous sodium chloride (150 mL). The organic phase was dried (Na_2SO_4), evaporated under reduced pressure and crystallized, on scratching, from 95% ethanol to give 70.6 g (59%) of **30** as colorless needles, m.p. 75-75.5°C (lit.[181] m.p. 75-76°C); IR ($CHCl_3$ cast) 1820 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.50 (m); exact mass 238.0630 (238.0630 calc. for $C_{15}H_{10}O_3$).

Preparation of 32 from reaction of 5-ethyl L-glutamate (29) with 30

The procedure of Sheehan and Guziec[181] was used. A mixture of 5-ethyl L-glutamate (**29**) (1.8 g, 10 mmol) and tetramethylammonium hydroxide (20% in methanol, 4.6 g, 10 mmol) was evaporated under reduced pressure and the residue was suspended in DMF (10 mL). The dioxolone **30** (2.4 g, 10 mmol) was added and the mixture was stirred 30 min at room temperature. Addition of 2 M hydrochloric acid (10 mL) and ethyl acetate (50 mL) was followed by washing of the mixture with water (3 x 40 mL). The organic phase was dried (Na_2SO_4) and evaporated in vacuo to give 2.3 g (96%) of **30** unchanged.

The aqueous phase was concentrated *in vacuo* and the residue was applied to an ion exchange column (AG 1-X8, 50-100 mesh, OH^- form, 100 mL bed volume) and eluted with 1 M hydrochloric acid to give 1.58 g of a viscous oil identified as crude 5-oxopyrrolidine-2-carboxylic acid (**32**). A small amount of this was recrystallized twice from ethanol/ether to give **32** as a white solid, m.p. 158-159°C (lit.[183] m.p. 162-163°C); IR (MeOH cast) 3400, 3340, 1710 (br) cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.45 (m, 1H, C*H*), 2.45 (m, 4H, CH_2); exact mass 129.0425 (129.0426 calc. for $C_5H_7NO_3$).

(*S*)-2-Amino-5-hydroxypentanoic acid[184] (33)

The procedure of Thompson *et al*[184] was used. Lithium borohydride (0.64 mol, 14 g) was added to a stirred suspension of 5-ethyl L-glutamate (**29**) (0.16 mol, 28 g) in THF (600 mL). The mixture was heated at reflux for 18 h, then was cooled to 15°C and 60% aqueous methanol (400 mL) was added slowly. The mixture was stirred at room temperature 2 h, acidified to pH 5 with glacial acetic acid and evaporated to dryness. The residue was taken up in water (100 mL), filtered and acidified to pH 2 with concentrated hydrochloric acid. The precipitate which formed was filtered and the filtrate was concentrated *in vacuo* to *ca.* 50 mL volume and filtered again. The filtrate was applied to an ion exchange column (AG50W-X8, 50-100 mesh, H^+ form, 425 mL bed volume) and eluted with 2 M aqueous ammonia. The eluate was evaporated under reduced pressure and the residue was

recrystallized from water/methanol to give 6.5 g (31%) of **33** as white crystals, m.p. 221°C (dec.), $[\alpha]_D$ = +6.13°, c = 6.2, H_2O (lit.[184] m.p. 216°C, $[\alpha]_D$ = +6.19°, c = 4, H_2O); IR (KBr) 3340, 3240, 1585 (br), 1525 cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 3.79 (dd, J = 6 Hz, 7 Hz, 1H, C*H*), 3.68 (t, J = 6 Hz, 2H, CH_2OH), 1.96 (m, 2H, CH_2CH), 1.66 (m, 2H, CH_2CH_2OH); MS (FAB) 267 (M_2H^+), 134 (MH^+).

(*S*)-2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-5-pentanolide (34)

The procedure of Sheehan and Guziec[181] was followed. A mixture of **33** (10 mmol, 1.3 g) and tetramethylammonium hydroxide (20% in methanol, 10 mmol, 4.6 g) was concentrated to dryness <u>in vacuo</u>. The solid residue was suspended with stirring in DMF (10 mL) and **30** (10 mmol, 2.4 g) was added. The mixture was stirred 30 min at room temperature and 2 M hydrochloric acid (10 mL) and ethyl acetate (50 mL) were added. The mixture was washed with water (3 x 40 mL) and the organic extract was dried (Na_2SO_4) and concentrated under reduced pressure. The foamy residue was dissolved in trifluoroacetic acid (10 mL), stirred 2 h at room temperature, and concentrated <u>in vacuo</u>. The residue was partitioned between dichloromethane (40 mL) and water (2 x 15 mL). The dichloromethane extract was washed with saturated aqueous sodium chloride (15 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was recrystallized from dichloromethane/ethyl acetate to give 1.70 g (51%) of

34 as a white crystalline solid, m.p. 229-230°C; $[\alpha]_D$ = -64.6°, c = 1.3, $CHCl_3$; IR (CH_2Cl_2 cast) 1750, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.54 (m, 5H, *Ph*), 7.26 (m, 5H, *Ph*), 4.37 (m, 2H, CH_2O), 4.22 (dd, J = 7.5 Hz, 11.5 Hz, 1H, *CH*), 2.52 (m, 1H, $H\text{-}3_{ax}$), 2.14 (m, 1H, $H\text{-}3_{eq}$), 1.95 (m, 2H, CH_2CH_2O); exact mass 335.1160 (335.1158 calc. for $C_{20}H_{17}NO_4$); Anal. Calc. for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11; N, 4.18. Found: C, 71.53; H, 5.24; N, 4.04.

Ethyl (*S*)-5-bromo-2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-pentanoate (35)

The procedure of Plieninger[185] was used. Anhydrous ethanol (10 mL) was saturated with hydrogen bromide gas at 4°C and **34** (1.0 g, 3.0 mmol) was added. The mixture was stirred at 4°C for 18 h, and was partitioned between water (40 mL) and dichloromethane (50 mL). The dichloromethane extract was washed with 10% aqueous potassium bicarbonate solution (40 mL) and the basic aqueous phase was extracted with dichloromethane (2 x 50 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and evaporated to dryness. The residue was purified by flash chromatography[230] (20% ethyl acetate/hexane) to give 1.25 g (94%) of **35** as an oil; $[\alpha]_D$ = -27.3°, c = 1.8, CH_2Cl_2; IR ($CHCl_3$ cast) 1765, 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.58 (m, 3H, *Ph*), 7.44 (m, 2H, *Ph*), 7.25 (s, 5H, *Ph*), 4.26 (q, J = 7 Hz, 2H, CH_3CH_2), 4.17 (dd, J = 6 Hz, 10 Hz, 1H, *CH*), 3.33 (dt, J = 1

Hz, 8 Hz, 2H, CH_2Br), 2.26 (m, 2H, CH_2CH), 1.87 (m, 2H, CH_2CH_2Br), 1.32 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 445.0714, 443.0726 (445.0712, 443.0732 calc. for $C_{22}H_{22}BrNO_4$); Anal. Calc. for $C_{22}H_{22}BrNO_4$: C, 59.47; H, 4.99; N, 3.15; Br, 17.98. Found: C, 59.69; H, 4.93; N, 2.78; Br, 18.01.

tert-Butyl N-(benzyloxycarbonyl)glycinate[187] (36)

The modified procedure of Anderson and Callahan[187] was followed. Isobutylene gas was passed rapidly for 1 h into a mixture of N-(benzyloxycarbonyl)glycine (63 g, 0.30 mol) and sulfuric acid (3 mL) in dichloromethane (600 mL). The reaction mixture was stoppered, stirred at room temperature 69 h, and was then washed with 0.23 M aqueous potassium bicarbonate (500 mL) and saturated aqueous sodium chloride (200 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to give 76.6 g (96%) of **36** as a colorless oil; IR ($CHCl_3$ cast) 3350, 1730 (br), 1525 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.34 (s, 5H, *Ph*), 5.38 (br s, 1H, *NH*), 5.12 (s, 2H, $PhCH_2$), 3.85 (d, J = 6 Hz, 2H, CH_2), 1.45 (s, 9H, $C(CH_3)_3$); exact mass 265.1319 (265.1314 calc. for $C_{14}H_{19}NO_4$).

tert-Butyl glycinate[187] (37)

The procedure of Anderson and Callahan[187] was followed with some modification. A solution of **36** (70 g, 0.26 mol) in methanol (100 mL) was added to 5% palladium on charcoal (2

g) moistened with methanol. The mixture was shaken under hydrogen at an initial pressure of 48 p.s.i. for 8 days and was filtered through a Celite pad and concentrated *in vacuo*. The residue was distilled under reduced pressure to give 21.4 g (63%) of 37 as a colorless liquid, b.p. 98-100°C at 85 mm Hg (lit.[187] b.p. 30°C at 2 mm Hg); IR ($CHCl_3$ cast) 3350 (br), 1740, 1665, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 3.33 (s, 2H, CH_2), 1.48 (s, 9H, $C(CH_3)_3$), 1.39 (s, 2H, NH_2); exact mass 131.0944 (131.0946 calc. for $C_6H_{13}NO_2$).

tert-Butyl N-(benzylidene)glycinate[186] (38)

The procedure of Oguri *et al*[186] was followed with slight modification. A mixture of 37 (1.0 g, 7.6 mmol), benzaldehyde (0.81 g, 7.6 mmol) and magnesium sulfate (1.0 g) in benzene (20 mL) was stirred 26 h at room temperature. The mixture was filtered and the filtrate was concentrated *in vacuo* to leave 1.58 g (95%) of 38 as an unstable, colorless, mobile oil. This was not purified further before use. For 38; IR ($CHCl_3$ cast) 1730, 1645, 1370, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.28 (br s, 1H, PhC*H*), 7.81 (m, 2H, *Ph*), 7.40 (m, 3H, *Ph*), 4.33 (s, 2H, CH_2), 1.50 (s, 9H, $C(CH_3)_3$); exact mass 219.1246 (219.1259 calc. for $C_{13}H_{17}NO_2$).

Ethyl (S)-5-hydroxy-2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-pentanoate (39)

A mixture of ethanol (50 mL) and sulfuric acid (1.8 g, 1.0 mL, 19 mmol) was cooled to 4°C and **34** (4.2 g, 12 mmol) was added, but did not completely dissolve. This was stirred 10 min at 4°C and was then warmed to room temperature and stirred 1 h. Dichloromethane (30 mL) was added to aid dissolution and stirring was continued at room temperature 53 h. The mixture was partitioned between water (50 mL) and dichloromethane (50 mL). The organic extract was washed with 10% aqueous potassium bicarbonate (30 mL), dried (Na_2SO_4), and concentrated *in vacuo* to leave a colorless oil. Flash chromatography[230] (30% ethyl acetate/ dichloromethane) gave 2.91 g (62%) of **39** as a colorless oil and 0.37 g (9%) of recovered **34**. For **39**; $[\alpha]_D$ = -31.7°, c = 3.0, CH_2Cl_2; IR ($CHCl_3$ cast) 3470 (br), 1760, 1750 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.45 (m, 3H, *Ph*), 7.33 (m, 2H, *Ph*), 7.13 (m, 5H, *Ph*), 4.17 (q, J = 7 Hz, 2H, CH_2CH_3), 4.10 (dd, J = 4.5, 10 Hz, 1H, *CH*), 3.52 (t, J = 6.5 Hz, 2H, CH_2OH), 2.15 (m, 2H, CH_2CH), 1.68 (br s, 1H, *OH*), 1.50 (m, 2H, CH_2CH_2OH), 1.23 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 381.1581 (381.1576 calc. for $C_{22}H_{23}NO_5$); Anal. Calc. for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 68.90; H, 6.08; N, 3.64.

Ethyl (S)-5-(p-tolylsulfonyloxy)-2-(2-oxo-4,5-diphenyl-1,3--oxazol-3-yl)pentanoate (40)

A mixture of **39** (2.0 g, 5.2 mmol), p-toluenesulfonyl chloride (1.1 g, 6.0 mmol) and 4-(dimethylamino)pyridine (0.73 g, 6.0 mmol) in dichloromethane (25 mL) was stirred at

room temperature 42 h. The mixture was poured into water (25 mL), the aqueous phase was extracted with dichloromethane (25 mL) and the combined organic phases were dried (Na_2SO_4) and evaporated under reduced pressure. Flash chromatography[230] (35% ethyl acetate/hexane) of the residue gave 1.31 g (47%) of **40** as a colorless oil and 0.36 g (17%) of an oil identified as ethyl (*S*)-5-chloro-2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)pentanoate (**41**). For **40**; $[\alpha]_D$ = -12.3°, c = 1.3, CH_2Cl_2; IR ($CHCl_3$ cast) 1760, 1360, 1175 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.69 (d, J = 8 Hz, 2H, H_{ortho} to SO_2), 7.50 (d, J = 8 Hz, 2H, H_{ortho} to CH_3), 7.5-7.1 (m, 10 H, *Ph*), 4.18 (q, J = 7 Hz, 2H, CH_2CH_3), 4.04 (dd, J = 6Hz, 10 Hz, 1H, *CH*COOEt), 3.92 (t, J = 6 Hz, 2H, CH_2OTs), 2.40 (s, 3H, CH_3), 2.06 (m, 2H, CH_2CHCOOEt), 1.62 (m, 2H, CH_2CH_2OTs), 1.22 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 535.1665 (535.1665 calc. for $C_{29}H_{29}NO_7S$); Anal. Calc. for $C_{29}H_{29}NO_7S$: C, 65.03; H, 5.46; N, 2.62; S, 5.99. Found: C, 64.93; H, 5.54; N, 2.54; S, 5.64. For **41**: $[\alpha]_D$ = -29.7°, c = 1.2, CH_2Cl_2; IR ($CHCl_3$ cast) 1760 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.48 (m, 3H, *Ph*), 7.35 (m, 2H, *Ph*), 7.16 (m, 5H, *Ph*), 4.20 (q, J = 7 Hz, 2H, CH_2CH_3), 4.11 (dd, J = 6 Hz, 9.5 Hz, 1H, *CH*COOEt), 3.41 (t, J = 6.5 Hz, 2H, CH_2Cl), 2.21 (m, 2H, CH_2CHCOOEt), 1.74 (m, 2H, CH_2CH_2Cl), 1.26 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 399.1238 (399.1237 calc. for $C_{22}H_{22}ClNO_4$). Anal. Calc. for $C_{22}H_{22}ClNO_4$: C, 66.08; H, 5.55; N, 3.50; Cl, 8.87. Found. C, 66.30; H, 5.61; N, 3.32; Cl, 9.13.

Ethyl (S)-5-iodo-2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)-pentanoate (42)

To a stirred suspension of magnesium turnings (0.15 g, 6.2 mmol) in anhydrous ether (10 mL) was added iodine (1.3 g, 5.0 mmol) and the mixture was stirred 30 min at room temperature, at which point the color of the iodine had disappeared. The magnesium iodide solution (4.0 mL, 2.0 mmol) was transferred to another flask and a solution of **40** (0.52 g, 0.97 mmol) in ether (4 mL) was added. Stirring was continued 30 min, the reaction was quenched by the addition of ice-water (10 mL) and the mixture was extracted with ether (3 x 20 mL). The combined ether extracts were decolorized by washing with 5% aqueous sodium thiosulfate (20 mL), dried (Na_2SO_4) and evaporated <u>in vacuo</u> to leave 397 mg (83%) of **42** as an unstable pale yellow oil; $[\alpha]_D$ = -22.5°, c = 4.4, CH_2Cl_2; IR ($CHCl_3$ cast) 1765, 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.55 (m, 3H, *Ph*), 7.40 (m, 2H, *Ph*), 7.20 (m, 5H, *Ph*), 4.22 (q, J = 7 Hz, 2H, CH_2CH_3), 4.11 (dd, J = 5.5, 10 Hz, 1H, *CH*COOEt), 3.04 (dt, J = 1.5, 7 Hz, 2H, CH_2I), 2.16 (m, 2H, $CH_2CHCOOEt$), 1.79 (m, 2H, CH_2CH_2I), 1.27 (t, J = 7 Hz, 3H, CH_2CH_3); exact mass 491.0598 (491.0594 calc. for $C_{22}H_{22}INO_4$).

Dimethyl 2,6-diaminoheptanedioate hydrochloride[188,189] (43)

A suspension of 2,6-diaminoheptanedioic acid (1) (Sigma, mixture of **LL**, **DD** and <u>meso</u> isomers) (10 g, 53 mmol) in methanol (400 mL) was stirred at 4°C while thionyl

chloride (18.8 g, 158 mmol) was added dropwise. When addition was complete, all of the solid had dissolved and stirring was continued 69 h at room temperature. Evaporation of the solvent in vacuo and recrystallization of the residue from methanol/ether gave 9.6 g (63%) of **43** as a white solid, m.p. 185-186°C (lit.[189] m.p. 186-187°C); IR ($MeOH/CHCl_3$ cast) 1745 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.20 (br t, 2H, C*H*), 3.88 (s, 6H, $COOCH_3$), 2.2-1.4 (m, 6H, CH_2); MS (FAB) 437 (M_2H^+), 219 (MH^+), 204 ($MH^+ - CH_3$), 142 ($M - COOCH_3, NH_3$).

Dimethyl 2-amino-6-(benzylamino)heptanedioate (44)

Triethylamine (1.7 g, 17 mmol) and benzaldehyde (1.8 g, 17 mmol) were added to a solution of **43** (5.0 g, 17 mmol) in methanol (30 mL) and the mixture was stirred 2 h at room temperature. Sodium borohydride (0.65 g, 17 mmol) was added and stirring was continued for 2 h. Evaporation of the solvent in vacuo left a pale yellow residue which was partitioned between dichloromethane (2 x 50 mL) and 10% aqueous potassium bicarbonate (40 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a pale yellow oil. Column chromatography (gradient elution with methanol/chloroform) afforded 1.44 g (27%) of **44** as a pale yellow oil and 1.24 g (18%) of a pale yellow oil identified as dimethyl 2,6-bis(benzylamino)-heptanedioate (**45**). For **44**: IR ($CHCl_3$ cast) 1730 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.32 (m, 5H, *Ph*), 3.82 (d, J = 12 Hz, 1H, PhC*H*HN), 3.73 (s, 3H, $COOCH_3$), 3.72 (s, 3H, $COOCH_3$),

3.61 (d, J = 12 Hz, 1H, PhCH*H*N), 3.45 (m, 1H, C*H*NH), 3.27 (t, J = 6 Hz, 1H, C*H*NH_2), 1.92 (br s, 3H, N*H*, NH_2), 1.58 (m, 6H, CH_2); exact mass 308.1729 (308.1736 calc. for $C_{16}H_{24}N_2O_4$); Anal. Calc. for $C_{16}H_{24}N_2O_4$: C, 62.32; H, 7.84; N, 9.08. Found: C, 62.13; H, 7.74; N, 8.89. For **45**: IR ($CHCl_3$ cast) 1750 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 7.34 (m, 10H, *Ph*), 3.85 (d, J = 13 Hz, 2H, PhC*H*HN), 3.73 (s, 6H, $COOCH_3$), 3.63 (d, J = 13 Hz, 2H, PhCH*H*N), 3.27 (m, 2H, C*H*), 1.9-1.4 (m, 8H, N*H*, CH_2); exact mass 398.2201 (398.2206 calc. for $C_{23}H_{30}N_2O_4$); Anal. Calc. for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.59; N, 7.03. Found: C, 68.99; H, 7.64; N, 6.99.

Dimethyl 2-benzylamino-6-(benzylideneamino)heptanedioate (46)

Benzaldehyde (0.17 g, 1.6 mmol) and anhydrous magnesium sulfate (0.5 g) were added to a solution of **44** (0.50 g, 1.6 mmol) in dichloromethane (10 mL) and the mixture was stirred at room temperature for 22 h. Removal of the magnesium sulfate by filtration and concentration of the filtrate in vacuo left 565 mg (88%) of **46** as an unstable colorless oil. This was not purified further before use. For **46**: IR ($CHCl_3$ cast) 1735, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 100 MHz) δ 8.32 (s, 1H, PhC*H*=N), 7.84 (m, 2H, *Ph*), 7.48 (m, 3H, *Ph*), 7.31 (s, 5H, *Ph*), 4.00 (m, 1H, C*H*N=), 3.76 (s, 3H, $COOCH_3$), 3.82 (d, J = 14 Hz, 1H, PhC*H*HN), 3.68 (s, 3H, $COOCH_3$), 3.62 (d, J = 14 Hz, 1H, PhCH*H*N), 3.28 (t, J = 6 Hz, 1H, C*H*NH), 2.2-1.2 (m incorporating s at 1.98, 7H, CH_2, N*H*); exact mass 396.2042

(396.2049 calc. for $C_{23}H_{28}N_2O_4$).

Dimethyl 2,6-bis(benzylamino)-2-difluoromethylheptanedioate (47)

The procedure of Bey et al[161] was followed, with slight modification. A stirred mixture of THF (10 mL), diisopropylamine (66 mg, 0.66 mmol) and HMPA (0.1 mL) was cooled to -78°C under argon atmosphere and butyllithium (0.96 M in hexane, 0.69 mL, 0.66 mmol) was added. Stirring was continued at -78°C for 45 min and a solution of 46 (260 mg, 0.66 mmol) in THF (7 mL) was added. The orange solution was warmed to 40°C, the argon inlet was closed and chlorodifluoromethane gas was passed rapidly into the solution until an attached balloon expanded. The solution, which had lightened in color, was stirred at 40-50°C for 1 h and saturated aqueous sodium chloride (20 mL) was added. Extraction with ether (2 x 50 mL), drying of the ether extract (Na_2SO_4) and concentration in vacuo gave a dark yellow oil. This was dissolved in methanol (10 mL) and to this solution was added sodium cyanoborohydride (76 mg, 1.2 mmol) and acetic acid (40 mg, 0.66 mmol). The mixture was stirred 30 h at room temperature and was then partitioned between 10% aqueous potassium bicarbonate (50 mL) and dichloromethane (2 x 50 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a pale yellow oil. Repeated chromatography (flash chromatography [230] (5% methanol/dichloromethane), column

chromatography (5% methanol/dichloromethane), flash chromatography (10% ethyl acetate/dichloromethane; 50% ethyl acetate/dichloromethane) and preparative TLC (30% ethyl acetate/hexane, repeated) gave 10 mg (3%) of **47** and 15 mg (5%) of 2-butyl 6-methyl 2,6-bis(benzylamino)-2-(difluoromethyl)heptanedioate (**48**). For **47**: IR ($CHCl_3$ cast) 1735, 1250, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.32 (m, 10H, *Ph*), 5.95 (t, J = 54 Hz, 1H, CHF_2), 3.84-3.58 (m, 10H, $PhCH_2$, $COOCH_3$), 3.27 (t, J = 6 Hz, 1H, *CH*), 2.34 (br s, 2H, *NH*), 1.95-0.70 (m, 6H, CH_2); ^{19}F NMR ($CDCl_3$, 84.7 MHz) δ -130.3 (ABX system, J_{HF} = 54 Hz, J_{FF} = 281 Hz); exact mass 448.2173 (448.2174 calc. for $C_{24}H_{30}F_2N_2O_4$). For **48**: IR ($CHCl_3$ cast) 1730, 1250, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.32 (m, 10H, *Ph*), 5.96 (t, J = 55 Hz, 1H, CHF_2), 4.18 (m, 2H, $COOCH_2$), 3.85-3.60 (m, 7H, $PhCH_2$, $COOCH_3$), 3.27 (m, 1H, *CH*), 2.39 (br s, 2H, *NH*), 1.95-0.70 (m, 6H, CH_2); ^{19}F NMR ($CDCl_3$, 84.7 MHz) δ -130.3 (ABX system, J_{HF} = 55 Hz, J_{FF} = 282 Hz); exact mass 490.2641 (490.2643 calc. for $C_{27}H_{36}F_2N_2O_4$).

2,6-Bis(benzyloxycarbonylamino)heptanedioic acid[190] (49)

The procedure followed was that of Wade *et al.*[190] A solution of 2,6-diaminoheptanedioic acid (**1**) (Sigma, mixture of **LL**, **DD**, and *meso* isomers) (25 g, 0.13 mol) in 2 M aqueous sodium hydroxide (325 mL) was stirred and cooled to 4°C while benzyl chloroformate (61 g, 0.36 mol) was added dropwise over 30 min. Vigorous stirring was continued at

room temperature for 2 h and the mixture was extracted with ethyl acetate (200 mL). The aqueous phase was acidified to pH 1 with 4 M aqueous hydrochloric acid and was extracted with ethyl acetate (3 x 250 mL). The ethyl acetate extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo* to leave 56.8 g (95% crude yield) of **49** as a foam. This was >95% pure by NMR and was used without further purification. For **49**: IR ($CHCl_3$/MeOH cast) 1720, 1535 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 10.27 (br s, 2H, COO*H*), 7.30 (s, 10H, *Ph*), 5.80 (br s, 2H, N*H*), 5.09 (s, 4H, PhCH_2O), 4.35 (m, 2H, C*H*COOH), 2.1-1.1 (m, 6H, CH_2); exact mass 306.1209 (306.1216 calc. for $C_{15}H_{18}N_2O_5$ (M - $PhCH_2OCO$, OH)).

A small sample (5 g) of **49** was recrystallized twice from ethyl acetate to give 1.51 g of white crystals, m.p. 166-166.5°C (lit.[190] m.p. 165.5°C for **DD-LL** mixture of isomers). The mother liquor from the first recrystallization was evaporated *in vacuo* and the residue was recrystallized from chloroform to leave a white solid, m.p. 124.5-126°C (lit.[190] m.p. 123.5-124.5°C for *meso* isomer).

Bis(*tert*-butyl) 2,6-bis(benzyloxycarbonylamino)heptanedioate[191] (50)

The procedure followed was that of Bricas *et al.*[191] A solution of **49** (9.2 g, 20 mmol) in dichloromethane (100 mL) in a pressure bottle was stirred and cooled to -78°C while concentrated sulfuric acid (30 drops) and isobutylene (90 mL condensed at -78°C, *ca.* 950 mmol) were added. The bottle was

sealed and the mixture stirred at room temperature for 67 h. The clear mixture was cooled to -78°C and the bottle was opened. The mixture was washed with 10% aqueous potassium bicarbonate (100 mL) and the aqueous phase was extracted with dichloromethane (2 x 50 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated *in vacuo* to leave 10.8 g (94%) of **50** as a viscous oil. This was >95% pure by NMR and was used without further purification, but a small analytical sample was obtained by flash chromatography[230] (ethyl acetate). For **50**: IR ($CHCl_3$ cast) 1725, 1525 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.34 (s, 10H, *Ph*), 5.30 (m, 2H, N*H*), 5.10 (s, 4H, PhCH_2), 4.23 (m, 2H, C*H*), 2.0-1.1 (m incorporating s at 1.44, 24H, CH_2, $C(CH_3)_3$); exact mass 514.2318 (514.2315 calc. for $C_{27}H_{34}N_2O_8$ (M - C_4H_8)).

Bis(*tert*-butyl) 2,6-diaminoheptanedioate[191] (51)

A: Hydrogenation at 1 atm pressure

A solution of **50** (8.55 g, 15.1 mmol) in methanol (75 mL) was added to 5% palladium on carbon (1 g) which had been premoistened with methanol. The mixture was stirred under hydrogen (atmospheric pressure) at room temperature for 4 days. The catalyst was removed by filtration through a Celite pad and the solvent was evaporated from the filtrate *in vacuo* to leave 4.5 g (98%) of **51** as an oil; IR (CH_2Cl_2 cast) 1730, 1370, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 3.35

(m, 2H, CH), 1.8-1.1 (m incorporating s at 1.45, 28H, NH_2, CH_2, $C(CH_3)_3$); exact mass 302.2213 (302.2206 calc. for $C_{15}H_{30}N_2O_4$).

B: Parr Hydrogenation

A solution of **51** (11.3 g, 19.9 mmol) in methanol (100 mL) was added to 5% palladium on carbon (1 g, premoistened with methanol) in a Parr bottle. The mixture was shaken under hydrogen atmosphere (initial pressure 49 p.s.i.) for 72 h. Removal of the catalyst by filtration through a Celite pad, followed by concentration of the filtrate in vacuo gave a pale brown oil. This was purified by column chromatography (Florisil, 60-100 mesh, gradient elution with chloroform/methanol) to give **51** (2.49 g, 41%) (spectroscopic data above) and an oil identified as bis(tert-butyl) 2-amino-6-(benzyloxycarbonylamino)heptanedioate (**52**) (1.29 g, 15%). An analytical sample of **52** was purified by bulb to bulb distillation (oven temperature 80-90°C, 0.05 mm Hg). For **52**: IR (CH_2Cl_2 cast) 1730, 1525, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.40 (s, 5H, Ph), 5.48 (br m, 1H, NH), 5.15 (s, 2H, $PhCH_2$), 4.27 (m, 1H, CHNH), 3.30 (m, 1H, $CHNH_2$), 1.9-1.1 (m incorporating s at 1.45, 26H, NH_2, CH_2, $C(CH_3)_3$); exact mass 436.2586 (436.2573 calc. for $C_{23}H_{36}N_2O_6$); Anal. Calc. for $C_{23}H_{36}N_2O_6$: C, 63.28; H, 8.31; N, 6.42. Found. C, 63.11; H, 8.33; N, 6.22.

Bis(tert-butyl) 2-amino-6-(benzylamino)heptanedioate (53)

Benzaldehyde (0.21 g, 2.0 mmol) was added to a solution of **51** (0.59 g, 2.0 mmol) in toluene (150 mL) and the mixture was heated to reflux 16 h through a Soxhlet apparatus containing a thimble filled with calcium hydride. Removal of the solvent *in vacuo* left a pale yellow oil, which was dissolved in methanol (10 mL). Sodium cyanoborohydride (0.25 g, 3.9 mmol) and acetic acid (0.23 g, 3.9 mmol) were added, the mixture was stirred at room temperature for 72 h, and was then partitioned between 10% aqueous potassium bicarbonate (20 mL) and dichloromethane (3 x 25 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The residual oil was purified by flash chromatography[230] (10% methanol/ethyl acetate) to give **53** (260 mg, 34%) as an oil and an oil identified as bis(*tert*-butyl) 2,6-bis(benzylamino)heptanedioate (**54**) (315 mg, 33%). For **53**: IR ($CHCl_3$ cast) 1730, 1370, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.32 (s, 5H, *Ph*), 3.88 (d, J = 14 Hz, 1H, PhC*H*HN), 3.62 (d, J = 14 Hz, 1H, PhCH*H*N), 3.4-3.0 (m, 2H, C*H*NH, C*H*NH_2), 1.9-1.2 (m incorporating singlets at 1.48 and 1.46, 27H, N*H*, NH_2, CH_2, $C(CH_3)_3$); exact mass 392.2673 (392.2675 calc. for $C_{22}H_{36}N_2O_4$); Anal. Calc. for $C_{22}H_{36}N_2O_4$: C, 67.32; H, 9.24; N, 7.14. Found: C, 67.45; H, 9.22; N, 7.15. For **54**: IR ($CHCl_3$ cast) 1730, 1370, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.33 (s, 10H, *Ph*), 3.86 (d, J = 13 Hz, 2H, PhC*H*HN), 3.62 (d, J = 13 Hz, 2H, PhCH*H*N), 3.12 (m, 2H, C*H*), 1.9-1.1 (m incorporating s at 1.45, 26H, N*H*, CH_2, $C(CH_3)_3$); exact mass 482.3145 (482.3145 calc. for $C_{29}H_{42}N_2O_4$); Anal.

Calc. for $C_{29}H_{42}N_2O_4$:: C, 72.17; H, 8.77; N, 5.80. Found: C, 72.15; H, 8.82; N, 5.67.

Bis(tert-butyl) 2,6-bis(benzylamino)-2-(difluoromethyl)-heptanedioate (55)

The procedure of Bey et al[161] was used with some modification for the first part of this reaction. Benzaldehyde (57 mg, 0.54 mmol) was added to a solution of 53 (212 mg, 0.540 mmol) in toluene (120 mL) and the mixture was heated to reflux 8 h through a Soxhlet apparatus containing a thimble filled with calcium hydride. The solvent was evaporated in vacuo and the residue was dissolved in THF (7 mL). This was added to a mixture of THF (5 mL), HMPA (0.05 mL), diisopropylamine (0.46 g, 4.5 mmol) and butyllithium (1.5 M in hexane, 0.30 mL, 0.45 mmol) which had been stirred at -78°C for 20 min under argon atmosphere. The resulting orange solution was warmed to 40°C and the argon inlet was closed while chlorodifluoromethane was passed rapidly into the solution until an attached balloon began to expand. The mixture lightened in color during the addition and stirring was continued for 1 h at 40-50°C. Saturated aqueous sodium chloride (20 mL) was added and the mixture was extracted with ether (3 x 25 mL). The combined ether extracts were dried (Na_2SO_4) and the solvent was removed in vacuo to leave a yellow oil. This was dissolved in methanol (5 mL) and sodium cyanoborohydride (58 mg, 0.90 mmol) and acetic acid (54 mg, 0.90 mmol) were added. The

mixture was stirred at room temperature for 19 h and was then partitioned between 10% aqueous potassium bicarbonate (20 mL) and dichloromethane (3 x 25 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo to leave a yellow oil. Purification by column chromatography (50% ethyl acetate/hexane) followed by preparative TLC (20% ethyl acetate/hexane) left 62 mg (26%) of **55** as an oil; IR ($CHCl_3$ cast) 1725, 1370, 1250, 1155 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.30 (m, 10H, *Ph*), 5.94 (t, J = 54 Hz, 1H, CHF_2), 3.86 (d, J = 13 Hz, 1H, $PhCHHN^6$), 3.83 (s, 2H, $PhCH_2N^2$), 3.61 (d, J = 13 Hz, 1H, $PhCHHN^6$), 3.15 (m, 1H, *CH*), 2.10 (br s, 2H, *NH*) 1.9-0.7 (m incorporating singlets at 1.49 and 1.46, 24H, CH_2, $C(CH_3)_3$); ^{19}F NMR ($CDCl_3$, 75.3 MHz) δ -125.3 (ABX system, J_{HF} = 54 Hz, J_{FF} = 281 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 174.9, 169.8, 140.7, 140.3, 128.5, 127.4, 117.5 (t, J_{CF} = 125 Hz), 82.8, 81.1, 67.3, 61.4, 52.3, 47.9, 34.0, 31.5, 28.5, 28.4, 19.6; exact mass 532.3114 (532.3113 calc. for $C_{30}H_{42}F_2N_2O_4$); Anal. Calc. for $C_{30}H_{42}F_2N_2O_4$: C, 67.65; H, 7.95; N, 5.26. Found: C, 68.03; H, 7.86; N, 5.10.

Bis(tert-butyl) 2,6-bis(benzylideneamino)heptanedioate (56)

Benzaldehyde (1.55 g, 14.7 mmol) was added to a solution of **51** (2.22 g, 7.34 mmol) in benzene (125 mL) and the mixture was heated at reflux with azeotropic removal of water for 2 h. Removal of the solvent in vacuo left 3.20 g (92%) of **56** as a pale yellow waxy solid which was used

without further purification. Recrystallization from hexane gave an analytical sample, m.p. 94-96°C; IR ($CHCl_3$ cast) 1730, 1640, 1370, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.51 (s, 2H, PhC*H*), 7.75 (m, 10H, *Ph*), 3.88 (br t, 2H, C*H*), 2.3-1.2 (m incorporating s at 1.46, 24H, CH_2, $C(CH_3)_3$); exact mass 478.2838 (478.2832 calc. for $C_{29}H_{38}N_2O_4$); Anal. Calc. for $C_{29}H_{38}N_2O_4$: C, 72.78; H, 8.00; N, 5.85. Found: C, 72.46; H, 7.90; N, 6.01.

Preparation of 55 from 56

A mixture of THF (7 mL), HMPA (0.1 mL) and diisopropylamine (0.68 g, 6.7 mmol) was stirred at -78°C under argon atmosphere while butyllithium (1.38 M in hexane, 4.9 mL, 6.7 mmol) was added slowly. The solution was stirred at -78°C for 30 min and a solution of **56** (3.2 g, 6.7 mmol) in THF (12 mL) was added. The dark red-orange solution was warmed to 40°C and the argon inlet was closed while chlorodifluoromethane was passed rapidly into the solution until an attached balloon expanded. The mixture was stirred at 40-50°C for 1 h during which time its color lightened appreciably. The mixture was partitioned between saturated aqueous sodium chloride (50 mL) and dichloromethane (3 x 50 mL) and the combined dichloromethane extracts were dried (Na_2SO_4) and concentrated <u>in vacuo</u>. The residual oil was dissolved in methanol (15 mL) and sodium cyanoborohydride (1.1 g, 17 mmol) and acetic acid (0:.805 g, 13.4 mmol) were added. The mixture was stirred at room temperature for 20 h,

and was then partitioned between 10% aqueous potassium bicarbonate (50 mL) and dichloromethane (3 x 50 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo to leave an orange oil. Column chromatography (20% ethyl acetate/ hexane) gave 555 mg (16%) of **55** (spectral data given above) and crude **57** (1.23 g). A portion of this (0.25 g) was purified by preparative TLC (10% ethyl acetate/ hexane) to leave an oil identified as bis(tert-butyl) 2,6-bis(difluoromethyl)-2,6-bis(benzylamino)heptanedioate (**57**) (0.22 g, 25%). For **57**; IR (CH_2Cl_2 cast) 1730, 1370, 1250, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.35 (m, 10H, *Ph*), 5.96 (t, J = 56 Hz, 2H, CHF_2), 3.83 (s, 4H, $PhCH_2$), 2.1-0.9, (m incorporating s at 1.48, 26H, *NH*, CH_2, $C(CH_3)_3$); ^{19}F NMR ($CDCl_3$, 75.3 MHz), -128.0 (ABX system, J_{HF} = 56 Hz, J_{FF} = 282 Hz), -128.1 (ABX system, J_{HF} = 56 Hz, J_{FF} = 282 Hz); exact mass 582.3086 (582.3081 calc. for $C_{31}H_{42}F_4N_2O_4$); Anal. Calc. for $C_{31}H_{42}F_4N_2O_4$: C, 63.90; H, 7.27; N, 4.81. Found. C, 64.28; H, 7.40; N, 4.92.

2,6-Diamino-2-(difluoromethyl)heptanedioic acid (3)

A mixture of THF (6 mL), HMPA (0.1 mL) and diisopropylamine (0.56 g, 5.5 mmol) was stirred and cooled to -78°C under argon atmosphere while butyllithium (1.38 M in hexane, 4.0 mL, 5.5 mmol) was added. Stirring was continued at -78°C for 30 min and a solution of **56** (2.6 g, 5.5 mmol) in THF (10 mL) was added. The dark orange solution was warmed to 40°C and the argon inlet was closed while

chlorodifluoromethane was passed rapidly into the solution until an attached balloon began to expand. Stirring was continued at 40-50°C for 1 h during which time the mixture lightened substantially in color. The solution was partitioned between saturated aqueous sodium chloride (50 mL) and dichloromethane (3 x 50 mL) and the combined dichloromethane extracts were dried (Na_2SO_4) and concentrated *in vacuo* to leave an orange oil (3.09 g). A major portion of this (2.5 g) was dissolved in glacial acetic acid (10 mL) and added to cooled (4°C) glacial acetic acid (10 mL) into which hydrogen bromide gas had been passed for 5-10 min. The mixture was immediately added to ice water (50 mL), then dichloromethane (50 mL) was added and the two-phase mixture was stirred vigorously at room temperature for 3 h. The phases were separated and the aqueous phase was washed with dichloromethane (2 x 50 mL). Concentration of the aqueous phase *in vacuo* left an orange foam. This was applied to a column of ion exchange resin (AG50W-X8, 50-100 mesh, H^+ form, 100 mL bed volume) and eluted with aqueous 1 M ammonia followed by water. The combined washings were concentrated *in vacuo*, reapplied to the column (regenerated in the H^+ form) and eluted with a linear concentration gradient of aqueous hydrochloric acid (1 M - 3 M). The fractions collected were monitored by ion-exchange TLC (Fixion 50-X8 plates, developed in 0.4 M sodium citrate buffer, pH 3.1)[342] and detected with ninhydrin spray. Concentration *in vacuo*

left 209 mg (19%) of **3** as a white solid, m.p. 223°C (dec.) (R_f = 0.64) and 156 mg (11%) of a solid identified as 2,6-bis(difluoromethyl)-2,6-diaminoheptanedioic acid (**63**) (R_f = 0.85). For **3**; IR (KBr) 2900 (br), 1750 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 6.47 (t, J = 54 Hz, 1H, CHF_2), 4.16 (t, J = 7 Hz, 1H, CH), 2.35-1.25 (m, 6H, CH_2); ^{19}F NMR (D_2O, 75.3 MHz) δ -127.0 ppm (ABX system, J_{HF} = 54 Hz, J_{FF} = 286 Hz); MS (FAB) 481 (M_2H^+), 333 (M glycerol H^+), 241 (MH^+), 178 (MH^+ - CO_2, HF); Anal. Calc. for $C_8H_{14}F_2N_2O_4 \cdot 2HCl \cdot 0.5\ H_2O$: C, 29.83; H, 5.32; N, 8,70. Found: C, 29.46; H, 5.24; N, 8.54. For **63**; IR (KBr) 3000 (br), 1750 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 6.45 (t, J = 54 Hz, 2H, CHF_2), 2.35-1.25 (m, 6H, CH_2); ^{19}F NMR (D_2O, 75.3 MHz) δ -126.2 ppm (ABX system, J_{HF} = 54 Hz, J_{FF} = 290 Hz); MS (FAB) 581 (M_2H^+), 383 (M glycerol H^+), 291 (MH^+), 228 (MH^+ - CO_2, HF); Anal. Calc. for $C_9H_{14}F_4N_2O_4 \cdot 0.5\ H_2O$: C, 36.13; H, 5.05; N, 9.36. Found: C, 36.22; H, 4.91; N, 9.24.

Reaction of 2,6-diaminoheptanedioic acid (1) with meso-diaminopimelate-D-dehydrogenase

A: Without catalase:

The procedure of White[47] was modified. A mixture of 2,6-diaminoheptanedioic acid (**1**) (Sigma, mixture of **DD**, **LL** and meso isomers) (0.76 g, 0.40 mmol), NADP (Sigma) (50 mg, 59 μmol), phenazine ethosulphate (10 mg, 30 μmol), dichlorophenolindophenol (0.32 g, 1.0 mmol) and

meso-diaminopimelate-D-dehydrogenase (1.6 units* in 0.40 mL of 10 mM potassium phosphate buffer, pH 7.4, containing 0.1% mercaptoethanol) in 0.25 M sodium carbonate buffer, pH 10.5 (100 mL) was allowed to stand at room temperature 43 h, with intermittent addition of more dehydrogenase (4.0 units total). The mixture was acidified to pH 1 with 5 M hydrochloric acid and applied to an ion exchange column (AG50W-X8, 50-100 mesh, H^+ form, 3 x 32 cm). Elution with 0.2 M hydrochloric acid (500 mL) and 1 M hydrochloric acid (1 L) gave a pale brown foam. To this was added a solution of hydroxylamine hydrochloride (0.28 g, 4.0 mmol) in 2 M hydrochloric acid (10 mL). The mixture was stirred 24 h at room temperature and was then applied to an ion exchange column (AG50W-X8, 50-100 mesh, H^+ form, 50 mL bed volume). Elution with 2% aqueous ammonia gave 18 mg (11%) of a solid identified as 2-aminoadipic acid[203] (65), m.p. 195°C (dec.) (lit.[203] m.p. 203-204°C for the L-isomer); IR (KBr) 3420, 3100 (br), 1640, 1510 cm^{-1}; ^1H NMR (D_2O, = 7 Hz, 1H, CH), 2.51 (t, J = 8 Hz, 2H, CH_2COOH), 2.05 (m, 2H, $CH_2CHCOOH$), 1.80 (m, 2H, CH_2); MS (FAB) 162 (MH^+).

B: With catalase:

The procedure of Misono et al[48] was used with modification. A mixture of 2,6-diaminoheptanedioic acid (1) (Sigma, mixture of DD, LL and meso isomers) (0.76 g, 0.40

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*1 unit is defined as the amount of enzyme which catalyses the formation of 1 μmol of NADPH per min at pH 10.5 and 25°C.[48]

mmol), NADP (Sigma) (0.84 g, 1.0 mmol), catalase (Sigma) (2 mg, 22,000 units)* and meso-diaminopimelate-D-dehydrogenase (0.5 units in 0.40 mL of 10 mM potassium phosphate buffer, pH 7.4, containing 0.1% mercaptoethanol) in 80 mM sodium carbonate buffer, pH 10.5 (100 mL) was allowed to stand at room temperature 136 h with intermittent addition of the dehydrogenase (3.0 units total) and catalase (12 mg total). The mixture was filtered through a Celite pad and then through an Amicon PM10 filter. The filtrate was applied to an ion exchange column (AG50W-X8, 50-100 mesh, H^+ form, 200 mL bed volume) and eluted with 0.2 M hydrochloric acid (500 mL) and 1 M hydrochloric acid (1 L). The ninhydrin-active fractions which did not contain diaminopimelic acid were combined and concentrated under reduced pressure to give a solid residue. This was dissolved in water (25 mL) and hydroxylamine hydrochloride (81 mg, 1.2 mmol) was added. The pH of the mixture was adjusted to 3 with 1 M sodium hydroxide and sodium cyanoborohydride (79 mg, 1.3 mmol) was added. The mixture was stirred at room temperature 20 h, acidified to pH 1 with 3 M hydrochloric acid and concentrated to dryness in vacuo. The residue was purified by ion exchange chromatography (AG50W-X8, H^+ form, 200 mL bed volume, elution with 2% aqueous ammonia) to give 91 mg (52%) of piperidine-2,5-dicarboxylic acid[206] (68). An analytical sample of this was converted to the hydrochloride

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*1 unit is defined as the amount of enzyme which catalyses the decomposition of 1 μmol of H_2O_2 per min at pH 7 and 25°C.

salt by treatment with 3 M hydrochloric acid and crystallization from 95% ethanol to give a white solid, m.p. 286-287°C (lit.[206] m.p. 287-289°C); IR (KBr) 3400, 3200 (br),1755, 1740 cm^{-1}; ^1H NMR (D_2O, 200 MHz) δ 3.80 (m, 2H, CHCOOH), 2.4-1.4 (m, 6H; CH_2); MS (FAB) 174 (MH^+).

Monoperoxyphthalic acid[208] (70)

The method of Payne[208] was followed, with slight modification. Hydrogen peroxide (30%, 1.8 g, 16 mmol) was added to a cooled (4°C), stirred solution of sodium carbonate (1.4 g, 13 mmol) in water (10 mL). Freshly recrystallized[343] phthalic anhydride (2.0 g, 13 mmol) was added and stirring was continued at 4°C for 30 min. The mixture was acidified to pH 1 with 17% sulfuric acid and extracted with ether (3 x 10 mL). The ether extract was washed with 40% aqueous ammonium sulfate (2 x 10 mL) and dried ($MgSO_4$) in the refrigerator. Aliquots (1.0 mL) of this solution were added to 20% potassium iodide (15 mL) and after 10 min, the iodine generated was titrated with 0.10 M sodium thiosulfate. The concentration of monoperoxyphthalic acid was thus measured to be 0.40 M (92% yield).

Di(tert-butyl) 2-(benzyloxycarbonylamino)-6-(hydroxylamino)-heptanedioate (71)

The procedure of Polonski and Chimiak[198] was followed. A mixture of 52 (1.3 g, 2.9 mmol), p-methoxybenzaldehyde (0.40 g, 2.9 mmol) and anhydrous sodium sulfate (1.0 g) in

dichloromethane (25 mL) was stirred 23 h at room temperature. The mixture was filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in ether (10 mL) and an ethereal solution of monoperoxyphthalic acid (70) (0.40 M, 10 mL, 4.0 mmol) was added. The mixture was left to stand in the refrigerator at 4°C for 4.5 h and was concentrated under reduced pressure. The residue was taken up in chloroform (*ca.* 20 mL) and left 13 h at 4°C. After filtration, the mixture was concentrated *in vacuo*. The residue was dissolved in 95% ethanol (10 mL) and hydroxylamine hydrochloride (0.28 g, 4.0 mmol) was added. The mixture was warmed gently to effect dissolution of the hydroxylamine hydrochloride and left 20 min at room temperature. After evaporation of the solvent *in vacuo*, the residue was purified by flash chromatography[230] (25% ether/dichloromethane; 60% ethyl acetate/hexanes) to give 590 mg (45%) of 71 as a colorless oil; IR ($CHCl_3$ cast) 3340, 1730 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.33 (s, 5H, *Ph*), 5.48 (d, J = 8 Hz, 1H, N*H*), 5.08 (s, 2H, PhCH_2), 4.23 (m, 1H, C*H*NH), 3.45 (m, 1H, C*H*NHOH), 1.9-1.1 (m including singlets at 1.47 and 1.44, 26H, $C(CH_3)_3$, CH_2, NHOH); exact mass 452.2526 (452.2522 calc for $C_{23}H_{36}N_2O_7$); Anal. Calc. for $C_{23}H_{36}N_2O_7$: C, 61.04; H, 8.02; N, 6.19. Found: C, 60.91; H, 7.84; N, 5.71.

2-Amino-6-(hydroxylamino)heptanedioic acid (4)

To a cooled (4°C) solution of 71 (0.54 g, 1.2 mmol) in glacial acetic acid (1 mL) was added a saturated solution of hydrogen bromide in acetic acid (1 mL). The mixture was allowed to warm to room temperature and, after 20 min, water (5 mL) was added. The mixture was concentrated in vacuo and the residue was dissolved in acetic acid (1 mL). The solution was cooled to 4°C and hydrogen bromide was bubbled through for 5-10 min. After 20 min further at 4°C, water (5 mL) was added and the mixture was applied to an ion exchange column (AG50W-X8, 50-100 mesh, H^+ form, 10 mL bed volume) and eluted with 2% aqueous ammonia to give 171 mg (69%) of 4 as an apparently chromatographically pure amorphous solid which reduced Fehling's solution[209]; IR (KBr) 3430 (br), 1630 (br) cm^{-1}; ^1H NMR (D_2O, 200 MHz) δ 3.76 (m, 1H, C*H*NHOH), 3.17 (m, 1H, C*H*NH_2), 1.42 (m, 2H, C*H*$_2$CHNHOH), 1.64 (m, 2H, C*H*$_2$CHNH_2), 1.46 (m, 2H, C*H*$_2$); MS (FAB) 207 (MH^+).

2-Benzyloxycarbonylamino)heptanedioic acid[222] (73)

The procedure of Augustin[222] was followed, with slight modification. A mixture of 2-aminoheptanedioic acid (Sigma) (10 g, 57 mmol) and 2 M aqueous sodium hydroxide (240 mL) was stirred and cooled to 4°C while benzyl chloroformate (12 g, 70 mmol) was added. Stirring was continued 22 h at room temperature and the mixture was washed with ethyl acetate (100 mL). The aqueous phase was brought to pH 2 with concentrated hydrochloric acid and extracted with ethyl

acetate (2 x 100 mL). The ethyl acetate extract was dried (Na_2SO_4) and concentrated in vacuo and the oily residue was crystallized from ethyl acetate/hexane to give 4.2 g (24%) of 73 as white crystals, m.p. 98-99°C (lit.[222] m.p. 97-98°C). The aqueous phase, upon standing, deposited 0.42 g (3%) of needles, m.p. 236-237°C, identified as 2-(benzylamino)heptanedioic acid (74). For 73; IR (CH_2Cl_2/MeOH cast) 3100 (br), 1710 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.92 (br s, 2H, COOH), 7.31 (s, 5H, Ph), 5.50 (m, 1H, NH), 5.11 (s, 2H, $PhCH_2$), 4.40 (m, 1H, CH), 2.34 (m, 2H, CH_2COOH), 2.0-1.1 (m, 6H, CH_2); exact mass 309.1209 (309.1212 calc. for $C_{15}H_{19}NO_6$). For 74; IR (KBr) 3600, 3380, 1710, 1630, 1590 cm^{-1}; ^1H NMR (D_2O/NaOD, 80 MHz) δ 7.32 (s, 5H, Ph), 3.95 (d, J = 14 Hz, 1H, PhCHH), 3.75 (d, J = 14 Hz, 1H, PhCHH), 3.25 (t, J = 6 Hz, 1H, CH), 2.07 (t, J = 7 Hz, 2H, CH_2COOH), 1.40 (m, 6H, CH_2); exact mass 265.1321 (265.1314 calc. for $C_{14}H_{19}NO_4$); Anal. Calc. for $C_{14}H_{19}NO_4 \cdot H_2O$: C, 59.35; H, 7.47; N, 4.94. Found, C, 59.05; H, 7.42; N, 4.94.

5-(3-Benzyloxycarbonyl-5-oxo-1,3-oxazolidin-4-yl)pentanoic acid (75)

The procedure of Itoh[223] was followed. Paraformaldehyde (2.0 g, 67 mmol) and p-toluenesulfonic acid (0.10 g, 0.53 mmol) were added to a stirred suspension of 73 (7.0 g, 23 mmol) in benzene (150 mL). The mixture was heated at reflux 2.5 h and was cooled and extracted with 10% aqueous potassium bicarbonate (4 x 50 mL). The aqueous extract was

acidified to pH 1 with concentrated hydrochloric acid and extracted with ethyl acetate (2 x 100 mL). The ethyl acetate extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography[230] (ethyl acetate) to give 6.60 g (89%) of 75 as a colorless oil. This was crystallized from ethyl acetate/hexanes; m.p. 64-65°C; IR (CH_2Cl_2 cast) 3100 (br), 1800, 1715 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 9.85 (br s, 1H, COO*H*), 7.37 (s, 5H, *Ph*), 5.55 (d, J = 5 Hz, 1H, OC*H*HN), 5.25 (d, J = 5 Hz, 1H, OCH*H*N), 5.22 (s 2H, PhCH_2), 4.35 (t, J = 5 Hz, 1H, C*H*), 2.35 (t, J = 7 Hz, 2H, CH_2COOH), 1.94 (m, 2H, CH_2CH), 1.56 (m, 4H, CH_2); exact mass 321.1209 (321.1212 calc. for $C_{16}H_{19}NO_6$); Anal. Calc. for $C_{16}H_{19}NO_6$: C, 59.81; H, 5.96; N, 4.36. Found: C, 59.74; H, 5.97; N, 4.34.

Methyl 5-(3-benzyloxycarbonyl-5-oxo-1,3-oxazolidin-4-yl)-pentanoate (76)

A: With Diazomethane

An ethereal solution of diazomethane was added to a solution of 75 (0.50 g, 1.6 mmol) in ether (20 mL) until a faint yellow color persisted. The solution was concentrated in vacuo and the residue was purified by flash chromatography[230] (40% ethyl acetate/hexanes) to give 0.41 g (76%) of 76 as a colorless oil; IR (CH_2Cl_2 cast) 1805, 1725 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.36 (s, 5H, *Ph*), 5.53 (d, J = 5 Hz, 1H, OC*H*HN), 5.23 (d, J = 5 Hz, 1H, OCH*H*N), 5.20 (s,

2H, $PhCH_2$), 4.33 (t, J = 5 Hz, 1H, CH), 3.66 (s, 3H, $COOCH_3$), 2.30 (t, J = 7 Hz, 2H, CH_2COOMe), 1.94 (m, 2H, CH_2CH), 1.52 (m, 4H, CH_2); exact mass 335.1369 (335.1369 calc. for $C_{17}H_{21}NO_6$); Anal. Calc. for $C_{17}H_{21}NO_6$: C, 60.89; H, 6.31; N, 4.18. Found: C, 60.69; H, 6.20; N, 4.01.

B: With Methyl Chloroformate

A solution of 75 (4.0 g, 12 mmol) in dichloromethane (100 mL) was stirred and cooled to 4°C while triethylamine (1.4 g, 14 mmol) and methyl chloroformate (1.3 g, 14 mmol) were added. The mixture was stirred at room temperature 1 h, methanol (1.6 g, 50 mmol) was added and stirring was continued 1 h. The mixture was washed with 10% aqueous potassium bicarbonate (50 mL) and the aqueous phase was extracted with dichloromethane (50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo, and the residue was taken up in methanol and concentrated again under reduced pressure. Flash chromatography[230] (40% ethyl acetate/hexane) of the residue gave 2.22 g (53%) of 76 (spectroscopic data above) and further elution of the column with ethyl acetate gave 1.36 g (34%) of recovered 75.

Reaction of 76 with sodium hydroxide

To a stirred solution of 76 (0.16 g, 0.48 mmol) in THF (1 mL) was added 1 M aqueous sodium hydroxide (1 mL). Stirring was continued 30 min at room temperature and the mixture was partitioned between 0.5 M aqueous hydrochloric acid (5 mL) and dichloromethane (2 x 10 mL). The

dichloromethane extract was dried (Na_2SO_4) and the solvent was evaporated *in vacuo* to leave 139 mg (94%) of 73 as a colorless oil which solidified at room temperature. This was spectroscopically and chromatographically identical with authentic 73.

tert-Butyl 5-(5-oxo-3-(benzyloxycarbonyl)-1,3-oxazolidin-4-yl)pentanoate (77)

The procedure followed was that of Itoh.[223] A solution of 75 (1.0 g, 3.1 mmol) and sulfuric acid (0.1 mL) in dichloromethane (20 mL) was cooled to -5-0°C and isobutylene gas was bubbled into the solution until an increase in volume of 15-20 mL was observed. The mixture was allowed to stand at room temperature 24 h, and was then partitioned between 10% aqueous potassium bicarbonate (10 mL) and dichloromethane (15 mL). The organic phase was washed with water (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. Flash chromatography[230] of the residue (30% ethyl acetate/hexanes) gave 0.25 g (21%) of 77 as a colorless oil; IR ($CHCl_3$ cast) 1800, 1720 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.37 (s, 5H, *Ph*), 5.54 (d, J = 5 Hz, 1H, OC*H*HN), 5.22 (d, J = 5 Hz, 1H, OCH*H*N), 5.20 (s, 2H, PhCH_2), 4.33 (t, J = 5 Hz, 1H, C*H*), 2.20 (t, J = 5 Hz, 2H, $CH_2COOC(CH_3)_3$), 2.1-1.1 (m including s at 1.42, 15H, CH_2, $C(CH_3)_3$); exact mass 378.1919 (378.1917 calc. for $C_{20}H_{28}NO_6$ (MH)); Anal. Calc. for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found. C, 63.69; H, 7.28; N, 3.47.

7-(tert-Butyl) hydrogen 2-(benzyloxycarbonylamino)heptanedioate (78)

To a stirred solution of 77 (0.18 g, 0.49 mmol) in THF (2 mL) was added 1 M aqueous sodium hydroxide (1 mL). Stirring was continued at room temperature 30 min, the mixture was acidified to pH 1 with 3 M hydrochloric acid and was then extracted with dichloromethane (2 x 10 mL). The dichloromethane extract was diluted with ethyl acetate and extracted with 10% aqueous potassium bicarbonate (4 x 10 mL) and the aqueous phase was acidified and extracted with ethyl acetate (6 x 20 mL). The ethyl acetate extract was dried (Na_2SO_4) and concentrated *in vacuo* to give 144 mg (80%) of 78. An analytical sample was recrystallized from ethyl acetate/hexanes to give white crystals, m.p.89-89.5°C; IR ($CHCl_3$ cast) 3330, 1725 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.53 (br s, 1H, COO*H*), 7.31 (s, 5H, *Ph*), 5.43 (br d, J = 6 Hz, 1H, N*H*), 5.11 (s, 2H, PhCH_2), 4.38 (m, 1H, C*H*), 2.20 (m, 2H, CH_2COO*t*-Bu), 2.0-1.1 (m including s at 1.42, 15 H, CH_2, C(CH_3)$_3$); exact mass 320.1862 (320.1862 calc. for $C_{18}H_{26}NO_4$ (M - COOH)); Anal. Calc. for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.32; H, 7.73; N, 4.03.

(*R*)-2-Amino-3-methyl-1-butanol[228] (81)

A modification of the procedure of Hsuno *et al*[228] was followed. A mixture of lithium aluminum hydride (19 g, 0.50 mol) and THF (1.5 L) was heated to reflux under argon atmosphere for 30 min, then D-valine (35 g, 0.30 mol) was

added in small portions. Heating of the mixture at reflux was continued 17 h, then Celite (20 g) was added followed by water (20 mL), 10% aqueous sodium hydroxide (20 mL) and water (60 mL). The mixture was filtered and the solid was washed with ethyl acetate (2 x 100 mL). The combined filtrates were concentrated in vacuo and the residue was distilled under reduced pressure to give 21.54 g (70%) of 81 as a colorless oil; b.p.60-63°C at 1.5 mm Hg (lit.[228] b.p. 55-57°C at 2 mm Hg); $[\alpha]_D$ = -14.4° (neat) (lit.[344] $[\alpha]_D$ = +14.6° (neat) for (S)-isomer); IR ($CHCl_3$ cast) 3200 cm^{-1} (br); ^1H NMR ($CDCl_3$, 200 MHz) δ 3.65 (dd, J = 4, 10.5 Hz, 1H, C*H*HOH), 3.31 (dd, J = 8.5, 10.5 Hz, 1H, CH*H*OH), 2.56 (ddd superimposed on a br s, J = 3.5, 6, 8.5 Hz, 4H, NH_2, O*H*, C*H*NH_2), 1.60 (dqq, J = 6.5, 6.5, 6.5 Hz, 1H, C*H*$(CH_3)_2$), 0.92 (2 overlapping d, J = 6.5, 6.5 Hz, 6 H, CH$(CH_3)_2$); exact mass 104.1080 (104.1075 calc. for $C_5H_{14}NO$ (MH)).

(*R*)-4-Isopropyl-1,3-oxazolidin-2-one[227] (82)

The modified procedure of Evans et al[227] was used. A solution of 81 (21 g, 0.20 mol) in toluene (600 mL) was stirred and cooled to 4°C while phosgene (24 g, 0.24 mol) was added by distillation. Triethylamine (25 g, 0.24 mol) was added and the thick slurry was heated at reflux 25 min. The mixture was cooled to 4°C, filtered, and the filtrate was concentrated in vacuo. Recrystallization of the residue from hexane gave 18.17 g (70%) of 82 as colorless crystals, m.p. 70-71°C, $[\alpha]_D$ = -14.2°. c = 7.1, $CHCl_3$ (lit.[227] m.p. =

71-72°C, $[\alpha]_D$ = +14.8°, c = 7.0, $CHCl_3$ for (S)-isomer); IR ($CHCl_3$ cast) 3270, 1750, 1730, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.26 (br s, 1H, NH), 4.47 (dd, J = 8.5, 8.5 Hz, 1H, CHHO), 4.13 (dd, J ; 8.5, 6.5 Hz, 1H, CHHO), 3.66 (ddd, J = 8.5, 6.5, 6.5 Hz, 1H, CHN), 1.67 (dqq, J = 6.5, 6.5, 6.5 Hz, 1H, $CH(CH_3)_2$), 0.97 (2 overlapping d, J = 6.5, 6.5 Hz, 6H, $CH(CH_3)_2$); exact mass 129.0790 (129.0790 calc. for $C_6H_{11}NO$).

3-(5-[3-Benzyloxycarbonyl-5-oxo-1,3-oxazolidin-4-yl]-pentanoyl)-(4*R*)-4-isopropyl-1,3-oxazolidin-2-one (83)

The method of Engel and Just[229] was used for the first part of this reaction. Oxalyl chloride (1.0 g, 8.0 mmol) was added to a stirred, cooled (4°C) solution of 75 (0.63 g, 2.0 mmol) in benzene (5 mL) and the mixture was stirred 40 min at room temperature. Concentration in vacuo left 564 mg (83%) of the crude bis(acid chloride). A portion of this (0.18 g, 0.51 mmol) was dissolved in THF (2 mL) and added to a stirred, cooled (4°C) mixture of **62** (67 mg, 0.52 mmol) and butyllithium (1.2 M in hexane, 0.43 mL, 0.51 mmol) in THF (5 mL) under argon atmosphere. The reaction was quenched by addition of 10% aqueous ammonium chloride (2 mL) and the mixture was extracted with dichloromethane (2 x 20 mL). The dichloromethane extract was dried (Na_2SO_4) and the solvent evaporated in vacuo to leave 135 mg (61%) of **83** as a colorless oil; IR ($CHCl_3$ cast) 1800, 1780, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.38 (s, 5H, Ph), 5.55 (d, J = 5 Hz, 1H, OCHHN), 5.25 (d, J = 5 Hz, 1H, OCHHN), 5.22 (s, 2H, $PhCH_2$),

4.30 (m, 4H, CH, CHN, CH_2O), 2.95 (t, J = 8 Hz, 2H, CH_2CO), 2.38 (m, 1H, $CH(CH_3)_2$), 2.2-1.1 (m, 6H, CH_2), 0.89 (2 overlapping d, J = 7 Hz, 7 Hz, 6H, $CH(CH_3)_2$); exact mass 432.1898 (432.1898 calc. for $C_{22}H_{28}N_2O_7$); Anal. Calc. for $C_{22}H_{28}N_2O_7$: C, 61.10; H, 6.53; N, 6.48. Found: C, 60.74; H, 6.15; N, 6.20.

Reaction of 83 with sodium hydroxide

To a stirred solution of 83 (96 mg, 0.22 mmol) in THF (2 mL) was added 1 M aqueous sodium hydroxide (1 mL). Stirring was continued 30 min at room temperature and the mixture was brought to pH 1 by addition of 3 M aqueous hydrochloric acid and extracted with dichloromethane (2 x 10 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated in vacuo to leave 94 mg (quantitative recovery) of a mixture of 73 and 62, spectroscopically (NMR) and chromatographically identical with authentic samples.

2-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioic acid (84)

The procedure of Sheehan and Guziec[181] was followed. A mixture of 2-aminoheptanedioic acid (Sigma) (7.0 g, 40 mmol) and tetramethylammonium hydroxide (20% in methanol, 36 g, 80 mmol) was concentrated to dryness under reduced pressure and the residue was suspended in DMF (40 mL) with stirring. To this mixture was added 30 (9.5 g, 40 mmol) and stirring was continued 40 min at room temperature. Aqueous 2 M hydrochloric acid was added and the mixture was extracted

with ethyl acetate (2 x 100 mL). The ethyl acetate extract was washed with water (3 x 30 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in trifluoroacetic acid (40 mL) and stirred 2 h at room temperature. After evaporation of the trifluoroacetic acid *in vacuo*, the residue was partitioned between dichloromethane (150 mL) and water (3 x 30 mL). The organic extract was dried (Na_2SO_4) and concentrated under reduced pressure. Crystallization of the residue from ethyl acetate/hexanes gave 8.74 g (55%) of **84** as white crystals, m.p. 132-132.5°C; IR ($CHCl_3$ cast) 3000 (br), 1760, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 10.63 (br s, 2H, COO*H*), 7.54 (m, 3H, *Ph*), 7.42 (m, 2H, *Ph*), 7.20 (m, 5H, *Ph*), 4.18 (dd, J = 4.5, 10.5 Hz, 1H, C*H*), 2.31 (m, 3H, CH_2COOH, C*H*HCHCOOH), 2.03 (m, 1H, CH*H*CHCOOH), 1.51 (m, 2H, CH_2CH_2COOH), 1.34 (m, 2H, CH_2); exact mass 395.1362 (395.1369 calc. for $C_{22}H_{21}NO_6$); Anal. Calc. for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.73; H, 5.31; N, 3.46.

3,3'-[(*2S*)-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(*4R*)-4-isopropyl-1,3-oxazolidin-2-one] (85)

The procedure of Engel and Just[229] was followed for the first part of this reaction. To a cooled (4°C) suspension of **84** (4.0 g, 10 mmol) in benzene (20 mL) was added oxalyl chloride (5.1 g, 40 mmol) and dichloromethane (20 mL). The mixture was allowed to warm to room temperature with stirring 1.5 h, and was then concentrated *in vacuo*. The

residue was added as a THF solution (15 mL) to a stirred, cooled (4°C) mixture of 82 (2.6 g, 20 mmol), butyllithium (1.16 M in hexane, 17 mL, 20 mmol) and THF (50 mL) under argon atmosphere. The reaction was quenched by addition of 10% aqueous ammonium chloride (20 mL) and the mixture was extracted with dichloromethane (3 x 50 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was fractionated by flash chromatography[230] (50% ethyl acetate/hexanes) to give 2.56 g (41%) of 85 as a colorless foam and 2.22 g (36%) of the (*2R*)-isomer (86) as a colorless foam. For 85; $[\alpha]^D$ = -11.4°, c = 4.5, CH_2Cl_2; IR ($CHCl_3$ cast) 1775, 1705 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.50 (m, 5 H, *Ph*), 7.20 (m, 5H, *Ph*), 5.24 (dd, J = 4, 10 Hz, 1H, C*H*), 4.43 (m, 2H, C*H*HO), 4.25 (m, 4H, CH*H*O, C*H*N), 2.91 (m, 2H, CH_2CO), 2.36 (m, 2H. $CH(CH_3)_2$), 1.91 (m, 2H, CH_2CHCO), 1.57 (m, 4H, CH_2), 0.89 (t, J = 7.5 Hz, 12 H, $CH(CH_3)_2$); exact mass 617.2741 (617.2737 calc. for $C_{34}H_{39}N_3O_8$); Anal. Calc. for $C_{34}H_{39}N_3O_8$: C, 66.11; H, 6.36; N, 6.80. Found: C, 66.29; H, 6.38; N, 6.51. For 86; $[\alpha]_D$ = -100.2°, c = 4.0, CH_2Cl_2; IR ($CHCl_3$ cast) 1780, 1700 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.52 (m, 3H, *Ph*), 7.43 (m, 2H, *Ph*), 7.22 (m, 5H, *Ph*), 5.19 (dd, J = 4, 10.5 Hz, 1H, C*H*), 4.41 (m, 2H, C*H*HO), 4.26 (m, 4H, CH*H*O, C*H*N), 2.92 (t, J = 6,5 Hz, 2H, CH_2CO), 2.45 (m, 2H, $CH(CH_3)_2$), 1.65 (m, 6H, CH_2), 0.86 (4 overlapping d, J = 7 Hz, 12 H, $CH(CH_3)_2$); exact mass 617.2726 (617.2737 calc. for $C_{34}H_{39}N_3O_8$); Anal. Calc. for $C_{34}H_{39}N_3O_8$: C, 66.11; H, 6.36; N, 6.80. Found: C,

66.17; H, 6.46; N, 6.52.

Dibenzyl 2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioate (87)

The modified procedure of Evans et al[226] was used. A cooled (4°C) mixture of benzyl alcohol (71 mg, 0.66 mmol), butyllithium (1.16 M in hexane, 0.56 mL, 0.65 mmol) and THF (2 mL) was added to a stirred, cooled (4°C) solution of 85 (0.20 g, 0.32 mmol) in THF (5 mL) under argon atmosphere. Stirring was continued 1 h at 4°C and the reaction was quenched by addition of 10% aqueous ammonium chloride (3 mL). The mixture was extracted with dichloromethane (2 x 20 mL) and the dichloromethane extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography[230] (30% ethyl acetate/hexanes; 50% ethyl acetate/hexanes) to give 80 mg (95%) of 82 and 146 mg (79%) of 87 as a colorless oil; $[\alpha]_D$ = -1.4°, c = 7.3, CH_2Cl_2; IR ($CHCl_3$ cast) 1760, 1740 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 7.32 (m, 20H, *Ph*), 5.20 (s, 2H, $PhCH_2$), 5.09 (s, 2H, $PhCH_2$), 4.20 (dd, J = 6, 10 Hz, 1H, *CH*), 2.33 (t, J = 7 Hz, 2H, CH_2COOCH_2Ph), 1.50 (m, 6 H, CH_2); exact mass 575.2298 (575.2308 calc. for $C_{36}H_{33}NO_6$); Anal. Calc. for $C_{36}H_{33}NO_6$: C, 75.11; H, 5.78; N, 2.43. Found: C, 75.07; H, 5.87; N, 2.43.

The (*2R*)-isomer (86) was treated in the same way to give a 63% yield of an oil chromatographically and spectroscopically identical with 87 except for its optical

rotation; $[\alpha]_D$ = +1.4°, c = 7.3, CH_2Cl_2.

2-Aminoheptanedioic acid (72)

A mixture of 87 (0.11 g, 0.18 mmol), 5% palladium on charcoal (33 mg) and 3 M aqueous hydrochloric acid (1.0 mL) in methanol (20 mL) was shaken under hydrogen (48 p.s.i.) 27 h at room temperature. The mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. The residue was purified by ion exchange chromatography (AG50W-X8, 50-100 mesh, H^+ form, 10 mL bed volume, eluted with 2% aqueous ammonia) to give 25 mg (79%) of 72; no measurable optical activity; IR (KBr) 3410, 3100 (br), 1630, 1580 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.04 (t, J = 6 Hz, 1H, *CH*COOH), 2.43 (t, J = 7 Hz, 2H, CH_2COOH), 1.96 (m, 2H, CH_2CHCOOH), 1.56 (m, 4H, CH_2); MS (FAB) 176 (MH^+).

3,3'-[(*2R*)-2-amino-heptanedioyl]-bis[(*4R*)-4-isopropyl-1,3-oxazolidin-2-one] hydrochloride (88)

A solution of 86 (0.50 g, 0.81 mmol) in methanol (50 mL) was added to 5% palladium on charcoal (0.10 g) moistened with 3 M aqueous hydrochloric acid (0.3 mL). The mixture was shaken under hydrogen (46 p.s.i.) for 94 h and was filtered through a Celite pad. The filtrate was concentrated and ether was added to induce crystallization of 88 as white needles, m.p. 167.5-168°C (169 mg). The mother liquor, on concentration, gave a further 105 mg of 88 (total yield, 274 mg, 78%); $[\alpha]_D$ = -86.9°, c = 1.2, CH_2Cl_2; IR ($CHCl_3$ cast)

2940 (br), 1780, 1700, 1390, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.90 (br s, 3H, NH_3^+), 5.21 (m, 1H, CHCO), 4.35 (m, 6H, CHN, CH_2O), 3.00 (m, 2H, CH_2CO), 2.59 (m, 1H, $CH(CH_3)_2$), 2.38 (m, 1H, $CH(CH_3)_2$), 2.08 (m, 2H, CH_2CHCO), 1.87 (m, 4H, CH_2), 0.93 (m, 12H, $CH(CH_3)_2$); exact mass 397.2209 (397.2213 calc. for $C_{19}H_{31}N_3O_6$ (free amine)); Anal. Calc. for $C_{19}H_{31}N_3O_6 \cdot HCl \cdot H_2O$: C, 50.49; H, 7.58; N, 9.30. Found: C, 50.61; H, 7.18; N, 9.33.

S,S'-Dibenzyl 2-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedithioate (89)

A solution of 86 (0.20 g, 0.32 mmol) in THF (2 mL) was added to a cooled (4°C), stirred mixture of benzyl thiol (80 mg, 0.65 mmol) and butyllithium (1.16 M in hexane, 0.56 mL, 0.65 mmol) in THF (5 mL) under argon atmosphere. Stirring was continued 15 min at 4°C and the reaction was quenched by addition of 10% aqueous ammonium chloride (5 mL). The mixture was extracted with dichloromethane (2 x 20 mL) and the dichloromethane extract was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography[230] (20% ethyl acetate/hexanes) to give 171 mg (88%) of 89 as a colorless oil; $[\alpha]_D$ = +4.3°, c = 5.8, CH_2Cl_2; IR ($CHCl_3$ cast) 1760, 1685 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.34 (m, 20H, Ph), 4.14 (s, 2H, $PhCH_2S$), 4.09 (m, 1H, CHCO), 4.06 (s, 2H, $PhCH_2S$), 2.48 (t, J = 8 Hz, 2H, CH_2CO), 2.10 (m, 2H, CH_2CHCO), 1.55 (CH_2CH_2CO), 1.24 (m, 2H, CH_2); exact mass 607.1858 (607.1851 calc. for $C_{36}H_{33}NO_4S_2$).

Hydrolysis of compound 89

To a stirred solution of **89** (0.11 g, 0.18 mmol) in ether (2 mL) was added 10% aqueous potassium bicarbonate (1 mL). Vigorous stirring was continued 40 min and THF (2 mL) was added to aid miscibility. After 10 min further, 1 M aqueous sodium hydroxide (0.50 mL) was added. Stirring was continued 18 h and 1 M aqueous sodium hydroxide (0.50 mL) was added. After 4 h further, the mixture was extracted with ether (2 x 10 mL). The aqueous phase was acidified to pH 2-3 with 0.5 M aqueous hydrochloric acid and extracted with ethyl acetate (2 x 10 mL). The ethyl acetate extract was dried (Na_2SO_4) and concentrated *in vacuo* to leave 34 mg (48%) of optically-inactive **84**, spectroscopically and chromatographically identical with authentic **84**.

2-(Acetamido)heptanedioic acid[190] (90)

The modified procedure of Wade *et al*[190] was used. To a mixture of 2-aminoheptanedioic acid (Sigma) (72) (15 g, 86 mmol) and 2 M aqueous sodium hydroxide (100 mL) was added acetic anhydride (9.6 g, 94 mmol) and 2 M aqueous sodium hydroxide (80 mL) in alternate portions. The mixture was stirred 1.5 h at room temperature, acidified to pH 1.5 with concentrated hydrochloric acid and was then concentrated *in vacuo*. The residue was dissolved in water and again concentrated *in vacuo*. Extraction of the residue with boiling acetone (150 mL) and concentration of the acetone extract under reduced pressure gave an oily residue which

was crystallized from acetone/ether to give 14.1 g (75%) of **90** as a white solid, m.p. 105-107°C. A small analytical sample was recrystallized from acetone/ether, m.p. 108-108.5°C (lit.[190] m.p. 111-112°C); IR (CH_2Cl_2/MeOH cast) 3100 (br), 1720, 1620, 1550 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.35 (t, J = 7 Hz, 1H, CH), 2.40 (t, J = 7 Hz, 2H, CH_2COOH), 2.02 (s, 3H, CH_3CONH), 1.6 (m, 6H, CH_2); exact mass 217.0947 (217.0950 calc. for $C_9H_{15}NO_5$).

(S)-2-Aminoheptanedioic acid[190] (91)

The modified procedure of Wade et al[190] was used. A solution of **90** (6.0 g, 28 mmol) in water (240 mL) was adjusted to pH 7-7.5 with 2 M aqueous lithium hydroxide. Hog renal acylase I (Sigma) (0.50 g, 417,500 units)* (EC 3.5.1.14) was added and the mixture was shaken 50 h at 39°C. Charcoal (2 g) was added to the mixture which was warmed to 40-50°C and filtered through a Celite pad. More charcoal (2 g) was added and the filtration process was repeated. The filtrate was concentrated in vacuo, the residue was dissolved in water (30 mL) and the pH was adjusted to 3-4 with 3 M aqueous hydrochloric acid. Concentration under reduced pressure, followed by addition of 95% ethanol (100 mL) induced crystallization of 0.90 g (37%) of **91**, m.p. 224-225°C; $[\alpha]_D$ = +17.3°C, c = 1.1, 5 M HCl (lit.[190] $[\alpha]_D$ = +21.5°C, c = 1, 5 M HCl); IR (KBr) 3420 (br), 3100 (br),

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*A unit is defined as the amount of enzyme which hydrolyses 1.0 μmol of N-acetyl-L-methionine per hour at pH 7.0 and 25°C.

1690 (br) cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 3.70 (m, 1H, C*H*), 2.23 (t, J = 7 Hz, 2H, CH_2COOH), 1.6 (m, 6H, CH_2); MS (FAB) 176 (MH^+).

(S)-2-(2-Oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioic acid (92)

The procedure of Sheehan and Guziec[181] was followed. A mixture of (S)-2-aminoheptanedioic acid (91) (0.53 g, 3.0 mmol) and tetramethylammonium hydroxide (20% in methanol, 2.8 g, 6.0 mmol) was concentrated to dryness in vacuo. The residue was suspended in DMF (3 mL) and **30** (0.72 g, 3.0 mmol) was added. Stirring was continued 30 min at room temperature and 2 M hydrochloric acid (5 mL) was added. The mixture was extracted with ethyl acetate (2 x 10 mL) and the ethyl acetate extract was washed with water (3 x 3 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was dissolved in trifluoroacetic acid (3 mL) and stirred 2 h at room temperature. After evaporation of the trifluoroacetic acid in vacuo, the residue was dissolved in dichloromethane (15 mL) and washed with water (3 x 3 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography[230] (1% acetic acid/ethyl acetate) to give 0.97 g (82%) of **92** as a colorless foam, $[\alpha]_D$ = -31.6°, c = 4.5, CH_2Cl_2; chromatographically and spectroscopically identical with the racemic material (**84**).

Alternative preparation of compound 85

The procedure of Engel and Just[229] was used for the first part of this reaction. Oxalyl chloride (0.26 g, 2.0 mmol) was added to a stirred, cooled (4°C) mixture of **92** (0.20 g, 0.50 mmol), benzene (3 mL) and dichloromethane (3 mL) and stirring was continued 1.5 h at room temperature. The mixture was concentrated in vacuo and the residue was dissolved in THF (2 mL). This was added to a stirred, cooled (4°C) mixture of **82** (0.13 g, 1.0 mmol), butyllithium (1.16 M in hexane, 0.87 mL, 1.0 mmol) and THF (5 mL). The reaction was quenched by addition of 10% aqueous ammonium chloride (3 mL) and the mixture was extracted with dichloromethane (2 x 20 mL). The dichloromethane extract was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The residue was fractionated by flash chromatography[230] (50% ethyl acetate/hexanes; 1% acetic acid/ethyl acetate) to give 45 mg (15%) of **85**, 70 mg (54%) of recovered **82** and 134 mg (67%) of recovered **92**. No trace of **86** could be detected in the reaction mixture. All compounds were spectroscopically and chromatographically identical with authentic samples.

3,3'-[(*2R*,*6S*)-2-(1,2-bis(benzyloxycarbonyl)hydrazino)-6--(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(*4R*)-4-isopropyl- 1,3-oxazolidin-2-one] (93)

A cooled (78°C) mixture of diisopropylamine (13 mg, 0.13 mmol), butyllithium (1.16 M in hexane, 0.12 mL, 0.13 mmol) and THF (5 mL) was stirred under argon atmosphere 30

min and **85** (83 mg, 0.13 mmol) was added as a THF solution (2 mL). Dibenzyl azodicarboxylate (48 mg, 0.16 mmol) was added as a THF solution (2 mL) and the reaction was quenched by addition of 10% aqueous ammonium chloride (2 mL). The mixture was allowed to warm to room temperature and was extracted with dichloromethane (2 x 20 mL). The dichloromethane extract was dried (Na_2SO_4) and concentrated *in vacuo* and the residue was purified by flash chromatography[230] (55% ethyl acetate/hexanes) to give 95 mg (80%) of **93** as a colorless foam; $[\alpha]_D$ = -14.0°, c = 7.7, CH_2Cl_2; IR ($CHCl_3$ cast) 3380, 3320, 1780, 1760, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 20H, *Ph*), 7.04 (s, 1H, *NH*), 5.82 (m, 1H, *H-6*), 5.21 (m, 5H, *H-2*, $PhCH_2$), 4.30 (m, 6H, *CH*N, CH_2O), 2.33 (m, 2H, $CH(CH_3)_2$), 1.85 (m, 6H, CH_2), 0.90 (m, 12H, $CH(CH_3)_2$); MS (FAB) 916 (MH^+); Anal. Calc. for $C_{50}H_{53}N_5O_{12}$: C, 65.56; H, 5.83; N, 7.65. Found: C, 65.46; H, 5.70; N, 7.43.

Dibenzyl 2-(1,2-bis(benzyloxycarbonyl)hydrazino)-6-(2-oxo--4,5-diphenyl-1,3-oxazol-3-yl)heptanedioate (94)

A cooled (4°C) mixture of benzyl alcohol (0.11 g, 1.0 mmol) and butyllithium (1.16 M in hexane, 0.79 mL, 0.92 mmol) in THF (2 mL) was added to a stirred, cooled (4°C) solution of **93** (0.42 g, 0.46 mmol) in THF (5 mL). Stirring was continued 1 h at 4°C and 10% aqueous ammonium chloride (3 mL) was added. The mixture was extracted with dichloromethane (2 x 25 mL) and the dichloromethane extract was

dried (Na_2SO_4) and concentrated *in vacuo*. The residue was fractionated by flash chromatography[230] (30% ethyl acetate/hexanes; 50% ethyl acetate/hexanes) to give 100 mg (84%) of **82** and 289 mg (72%) of **94** as a colorless oil; IR ($CHCl_3$) cast 3300, 1760, 1750 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 30H, *Ph*), 6.88 (s, 1H, N*H*), 5.16 (m, 6H, PhCH_2), 4.90 (m, 1H, *H-6*), 4.68 (s, 2H, PhCH_2OOC), 4.12 (m, 1H, *H-2*), 2.4-1.5 (m, 6H, CH_2); MS (EI) 873 (M^+); Anal. Calc. for $C_{52}H_{47}N_3O_{10}$: C, 71.46; H, 5.42; N, 4.81. Found: C, 71.54; H, 5.62; N, 4.45.

3,3'-[(*2R*,*6S*)-2-(1,2-bis(*tert*-butoxycarbonyl)hydrazino)--6-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioyl]-bis-[(*4R*)-4-isopropyl -1,3-oxazolidin-2-one] (95)

A mixture of diisopropylamine (80 mg, 0.81 mmol) and butyllithium (1.16 M in hexane, 0.70 mL, 0.81 mmol) in THF (5 mL) was cooled to -78°C and stirred 30 min under argon atmosphere. A solution of **85** (0.50 g, 0.81 mmol) in THF (3 mL) was added, followed by a solution of bis(*tert*-butyl) azodicarboxylate[233] (prepared by L.A. Trimble) (0.19 g, 0.81 mmol) in THF (2 mL). The reaction was quenched by addition of 10% aqueous ammonium chloride (2 mL) and the mixture was warmed to room temperature and extracted with dichloromethane (2 x 25 mL). The dichloromethane extract was dried Na_2SO_4) and concentrated *in vacuo*. The residue was fractionated by flash chromatography[230] (50% ethyl acetate/hexanes; 5% ether/dichloromethane) to give 107 mg

(21%) of the starting material **85** and 314 mg (46%) of **95** as a colorless foam. For **95**; $[\alpha]_D$ -11.2°, c = 1.2, CH_2Cl_2; IR ($CHCl_3$ cast) 3390, 1780, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.46 (m, 5H, *Ph*), 7.16 (m, 5H, *Ph*), 6.62 (br s, 1H, *NH*), 5.69 (m, 1H, *H-6*), 5.28 (m, 1H, *H-2*), 3.29 (m, 6H, CH_2O, CHN), 2.30 (m, 3H, $CH(CH_3)_2$, $CHHCH_2$), 1.82 (m, 5H, CH_2, $CHHCH_2$), 1.42 (s, 18H, $C(CH_3)_3$), 0.88 (m, 12H, $CH(CH_3)_2$); MS (EI) 847 (M^+); Anal. Calc. for $C_{44}H_{57}N_5O_{12}$: 62.32; H, 6.78; N, 8.26. Found: C, 62.05; H, 6.56; , 7.94.

Dibenzyl 2-[1,2-bis(tert-butoxycarbonyl)hydrazino]-6-(2-oxo-4,5-diphenyl-1,3-oxazol-3-yl)heptanedioate (96)

To a cooled (4°C), stirred mixture of benzyl alcohol (0.13 g, 1.2 mmol) and butyllithium (1.16 M in hexane, 1.0 mL, 1.2 mmol) in THF (2 mL) under argon atmosphere was added a cooled (4°C) solution of **95** (0.51 g, 0.60 mmol) in THF (2 mL). Stirring was continued 1 h at 4°C and the reaction was quenched by addition of 10% aqueous ammonium chloride (3 mL). The mixture was extracted with dichloromethane (2 x 25 mL) and the dichloromethane extract was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography[230] (30% ethyl acetate/hexanes) to give 372 mg (77%) of **96** as a colorless oil; IR ($CHCl_3$ cast) 3330, 1760, 1745, 1710 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 20H, *Ph*), 6.39 (s, 1H, *NH*), 5.12 (m, 3H, *H-6*, $PhCH_2$), 4.64 (s, 2H, $PhCH_2$), 4.11 (m, 1H, *H-2*),

2.6-1.0 (m including singlets at 1.40 and 1.35 , 24 H, CH_2, $C(CH_3)_3$); MS (EI) 805 (MH^+); Anal. Calc for $C_{46}H_{51}N_3O_{10}$: C, 68.56; H,6.38; N, 5.21. Found: C, 68.55; H, 6.44; N, 4.69.

2-Amino-6-hydrazinoheptanedioic acid dihydrochloride (5)

A mixture of **94** (0.22 g, 0.26 mmol), 5% palladium on charcoal (0.10 g) and 3 M hydrochloric acid (2 mL) in methanol (20 mL) was shaken under hydrogen (47 p.s.i.) 24 h at room temperature and was then filtered through a Celite pad. The filtrate was concentrated in vacuo and the residue was purified by ion exchange chromatography (AG50W-X8, 50-100 mesh, H^+ form, 10 mL bed volume, eluted with 1 M hydrochloric acid) to give 45 mg (62%) of 5 as an apparently chromatographically pure hygroscopic foam; IR (KBr) 3430 (br), 3100 (br), 1730, 1620 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.13 (m, 1H, $CHNHNH_2$), 3.88 (m, 1H, $CHNH_2$), 2.2-1.1 (m, 6H, CH_2); MS (FAB) 242 (MH^+ + HCl); Anal. Calc. for $C_7H_{15}N_3O_4 \cdot 2HCl \cdot 3H_2O \cdot CO_2$: C, 25.54; H, 6.16; N, 11.17. Found: C, 25.73; H, 6.50; N, 11.25.

Methyl L-lysinate dihydrochloride[235,236] (98)

A suspension of L-lysine hydrochloride (20 g, 0.11 mmol) in methanol (250 mL) was stirred and cooled to 4°C while thionyl chloride (20 g, 0.16 mmol) was added dropwise. Stirring was continued 28 h at room temperature, and the mixture was concentrated in vacuo to leave a quantitative

yield (26 g) of **98** as a white solid, m.p. 208°C (dec.) (lit.[235] m.p. 212°C (dec.)); $[\alpha]_D$ = +16.0°, c = 5.0, H_2O (lit.[236] $[\alpha]_D$ = +17.0°, c = 5.0, H_2O); IR (CH_2Cl_2 cast) 2940 (br), 1735 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 4.22 (t, J = 6 Hz, 1H, C*H*), 3.90 (s, 3H, $COOCH_3$), 3.08 (br t, J = 7 Hz, 2H, CH_2N), 2.2-1.3 (m, 6H, CH_2); MS (FAB) 175 (MCH_3^+), 161 (MH^+), 146 ($MH^+ - CH_3$), 144 ($MH^+ - NH_3$), 84 ($M - COOCH_3, NH_3$).

Methyl N^2,N^6-bis(benzylidene)lysinate[161] (99)

The procedure of Bey et al was used.[161] To a suspension of **98** (5.0 g, 21 mmol) in dichloromethane (100 mL) was added benzaldehyde (4.5 g, 43 mmol) and triethylamine (4.3 g, 43 mmol). The mixture was stirred 65 h at room temperature and was concentrated in vacuo. The residue was suspended in ether (100 mL), filtered and the filtered solid was washed with ether (100 mL). The filtrate was washed with water (50 mL) and saturated aqueous sodium chloride (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure to leave 6.4 g (89%) of **99** as a pale yellow oil; IR ($CHCl_3$ cast) 1740, 1640 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.29 (br s, 2H, PhC*H*), 7.75 (m, 4H, *Ph*), 7.38 (m, 6H, *Ph*), 4.02 (t, J = 7 Hz, 1H, C*H*), 3.72 (s, 3H, $COOCH_3$), 3.62 (t, J = 7 Hz, 2H, CH_2N), 2.3-1.1 (m, 6H, CH_2); exact mass 336.1832 (336.1838 calc. for $C_{21}H_{24}N_2O_2$).

Methyl 2,6-diamino-2-methylhexanoate dihydrochloride (100)

The procedure of Bey and Vevert[237] was followed. A mixture of diisopropylamine (1.8 g, 18 mmol) in THF (100 mL) was stirred and cooled to -78°C under argon atmosphere while butyllithium (1.38 M in hexane, 18 mmol, 13 mL) was added. The mixture was stirred at -78°C for 20 min, then **99** (6.0 g, 18 mmol) was added as a THF solution (15 mL). Methyl iodide (7.6 g, 54 mmol) was added and the mixture was allowed to warm to room temperature and to stir 4 h. The mixture was partitioned between water (50 mL) and ether (2 x 50 mL), and the organic extract was dried (Na_2SO_4) and concentrated <u>in vacuo</u> to leave a pale orange oil identified as crude methyl 2,6-bis(benzylideneamino)-2-methylhexanoate; IR ($CHCl_3$ cast) 1730, 1645 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.25 (s, 2H, PhC*H*), 7.70 (m, 4H, *Ph*), 7.41 (m, 6H, *Ph*), 3.63 (m incorporating s at 3.70, 5H, $COOCH_3$, CH_2N), 2.4-1.0 (m incorporating s at 1.50, 9H, CH_3, CH_2); exact mass 291.1857 (291.1862 calc for $C_{20}H_{23}N_2$ (M - $COOCH_3$)). This was taken up in ether (50 mL) and 2 M hydrochloric acid (50 mL), and the two-phase mixture was stirred vigorously at room temperature for 14 h. The phases were separated and the aqueous layer was washed with ether (50 mL) and concentrated under reduced pressure to leave 4.05 g (91%) of **100** as a foam. A small analytical sample was recrystallized from methanol/ether in 90% yield to give a white solid, m.p. 174°C (dec); IR (CH_2Cl_2 cast) 2960 (br), 1745 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ 3.90 (s, 3H, $COOCH_3$), 3.06 (t, J = 6 Hz, 2H, CH_2N) 2.2-1.1

(m incorporating s at 1.63, 9H, CH_3, CH_2); MS (FAB) 349 (M_2H^+), 335 ($M_2H^+ - CH_3$), 175 (MH^+), 160 ($MH^+ - CH_3$), 98 (M - $COOCH_3$, NH_3); Anal. Calc. for $C_8H_{18}N_2O_2 \cdot 2HCl$: C, 38.88; H, 8.16; N, 11.33. Found: C, 38.94; H, 8.18; N, 11.26.

2,6-Diamino-2-methylhexanoic acid [238] (101)

A mixture of **100** (3.9 g, 16 mmol) amd 6 M aqueous hydrochloric acid (100 mL) was heated at reflux for 18 h. The solvent was removed under reduced pressure and the residue was purified by ion exchange chromatography (AG50W-X8, 50-100 mesh, H^+ form, eluted with 1 M ammonia). Recrystallization twice from methanol/ether/98% ethanol gave 1.51 g (59%) of **101** as a solid, m.p. 208-209°C (dec). A small sample was transformed to the hydrochloride salt by by treatment with 2 M hydrochloric acid. Recrystallization from 98% ethanol/ether left a white solid, m.p. 278-279°C (dec.) (lit.[238] m.p. 266°C for monohydrochloride monohydrate); IR (KBr) 2900 (br), 1600 cm^{-1} (br); ^1H NMR (D_2O, 200 MHz) δ 2.97 (t, J = 7 Hz, 2H, CH_2N), 2.0-1.1 (m incorporating s at 1.37, 9H, CH_3, CH_2); MS (FAB) 321 (M_2H^+), 161 (MH^+), 98 (M - COOH, NH_3).

(*2S*)-Methyl 2,6-bis([(*1S*,*4S*)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo[2.2.1]heptane-1-carbonyl]amino)hexanoate (102)

To a cooled (4°C), stirred suspension of **98** (0.20 g, 0.86 mmol) in dichloromethane (5 mL) was added (-)-camphanoyl chloride (Aldrich) (0.56 g, 2.6 mmol) and

triethylamine (0.51 g, 5.0 mmol). Stirring was continued 2 h at 4°C and 2 h at room temperature. The mixture was washed with 10% aqueous potassium bicarbonate (5 mL) and the aqueous phase was extracted with dichloromethane (5 mL). The combined dichloromethane extracts were dried (Na_2SO_4) and concentrated in vacuo. Purification of the residue by flash chromatography[230] (ethyl acetate) gave 316 mg (70%) of **102** as a colorless oil; $[\alpha]_D$ = -16.8°, c = 1.2, CH_2Cl_2; IR ($CHCl_3$ cast) 3370, 1790, 1740, 1670, 1530 cm^{-1}); ^1H NMR (C_6D_6, 400 MHz) δ 7.30 (d, J = 8 Hz, 1H, N*H*), 6.78 (t, J = 6 Hz, 1H, N*H*), 4.73 (m, 1H, C*H*), 3.44 (s, 3H, COOCH_3), 3.26 (m, 1H, H_S), 3.11 (m, 1H, H_R), 2.45 (m, 2H, *H-6'$_{endo}$*), 1.9-1.2 (m, 12H, *H-6'$_{exo}$*, CH_2), 1.0-0.8 (6s, 18H, CH_3); ^{13}C NMR (C_6D_6, 100.6 MHz) δ 177.7, 177.4, 172.0, 167.2, 166.9, 92.4, 92.2, 55.14, 55.11, 53.7, 53.6, 52.1, 51.9, 38.8, 31.7, 30.6, 29.4, 29.13, 29.07, 23.2, 16.72, 16.69, 16.60, 9.7; exact mass 520.2789 (520.2785 calc. for $C_{27}H_{40}N_2O_8$), Anal. Calc.for $C_{27}H_{40}N_2O_8$: C, 62.29; H, 7.74; N, 5.38. Found: C, 62.63; H, 7.73; N, 5.05.

Decarboxylation of 1 in D_2O to form [6-^{2}H]-L-Lysine.

This was performed by Dr. M.M. Palcic. In a typical experiment, 0.2 units of wheat germ or 1.4 units of *B. sphaericus* *meso*-diaminopimelate decarboxylase were dissolved in 10 mM potassium phosphate buffer, pD 6.8 (25 mL) (pD is the uncorrected pH meter reading), containing 0.01% 2-mercaptoethanol and 0.1 mM pyridoxal phosphate. This

solution was concentrated to 2-3 mL in an Amicon ultrafiltration cell equipped with a PM-10 membrane, then resuspended in fresh buffer (25 mL). After a second concentration, the enzyme solution was added to 20 mM potassium phosphate buffer, pD 6.8 (50 mL), containing 2,6-diaminoheptanedioic acid (500 mg) (a mixture of DD, LL and meso isomers), from which exchangeable protons had been replaced by deuterium through repeated evaporation and dissolution in D_2O. To this was added 0.1 mM pyridoxal phosphate and 0.01% 2-mercaptoethanol and the reaction flask was sealed and left at room temperature (26°C) for 100 h. Lysine and unreacted 2,6-diaminoheptanedioic acid were removed from the enzyme by ultrafiltration and applied to an ion exchange column (AG 50W-X8, 100-200 mesh, H^+ form, 2 x 15 cm) equilibrated with water. The column was washed with water, then 1 M HCl to separate 2,6-diaminoheptanedioic acid from lysine. Fractions containing lysine (detected by TLC on Fixion 50X8 plates developed in 0.07 M sodium citrate buffer, pH 5.1 and sprayed with ninhydrin) were combined and concentrated in vacuo. The residue was used directly in the derivatisation reaction.

[6-^{2}H] (2*S*)-Methyl 2,6-bis ([(1*S*,4*S*)-4,7,7-trimethyl-3-oxo--2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)hexanoate (103)

The procedure followed was that of Armarego et al.[255] A mixture of [6-^{2}H]-L-lysine (from incubation with the wheat germ decarboxylase) (60 mg, 0.33 mmol), 2 M aqueous sodium

hydroxide (0.33 mL) and 3 M aqueous sodium hydroxide (0.33 mL) was added to a solution of (-)-camphanoyl chloride (0.23 g, 1.0 mmol) in toluene (0.33 mL). The mixture was stirred 2 h at room temperature with intermittent addition of 2 M sodium hydroxide to maintain the pH of the mixture above 7. The solution was washed with dichloromethane (2 mL), and the aqueous phase was acidified to pH 1 with 2 M hydrochloric acid and extracted with dichloromethane (2 x 5 mL). The second dichloromethane extract was dried (Na_2SO4) and concentrated in vacuo. The residue was dissolved in ether (10 mL) and an ethereal solution of diazomethane was added until a persistent yellow color was observed. Evaporation of the solvent in vacuo and purification of the residue by flash chromatography[230] (70% ethyl acetate/hexanes) gave 50 mg (30%) of **103** as a colorless oil; IR ($CHCl_3$ cast) 3370, 1790, 1745, 1670, 1530 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) identical to that of **102**, except for the loss of the multiplet at δ 3.11 (H_R); The samples from both sources had spectral and chromatographic ^{13}C NMR (C_6D_6, 100.6 MHz) identical to that of **102**, except for the loss of the signal at δ 38.8 and the introduction of a triplet at δ 38.4; exact mass 521.2844 (521.2847 calc. for $C_{27}H_{39}DN_2O_8$). The compound obtained by treating the [6-^2H]-L-lysine obtained from incubation of the Bacillus sphaericus decarboxylase in the manner described above gave identical spectroscopic and chromatographic properties.

Also isolated was 76 mg (36% based on (-) camphanoyl chloride) of white crystals identified as (*1S,4S*) methyl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate[257] (**104**). This was recrystallized from benzene/hexane to give colorless needles, m.p. 106°C, $[\alpha]_D$ = -13.5°, c = 2.2, EtOH (lit.[257] m.p. = 108.4-108.5°C, $[\alpha]_D$ = -12.4°, c = 2.17, EtOH); IR ($CHCl_3$ cast) 1780, 1725 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 3.87 (s, 3H, $COOCH_3$), 2.7-1.5 (m, 4H, CH_2), 1.12 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 0.97 (s, 3H, CH_3); exact mass 212.1050 (212.1049 calc. for $C_{11}H_{16}O_4$).

[2,6-$^2H_2$]-2,6-Diaminoheptanedioic acid[64] (105)

The modified procedure of Fujihara and Schowen[261] was followed. A mixture of 2,6-diaminoheptanedioic acid (Sigma, mixture of DD, LL and meso isomers) (5.0 g, 26 mmol), potassium hydroxide (5.90 g, 105 mmol) and pyridoxal hydrochloride (0.53 g, 2.6 mmol) were dissolved in D_2O (25 mL) and the mixture was stirred at room temperature for 9.5 h. The D_2O was removed by lyophilization and the residue was dissolved in fresh D_2O (35 mL). The mixture was heated at reflux 2 h, cooled, and acidified to pH 5 with concentrated hydrochloric acid and 95% ethanol was added until a permanent cloudiness was observed. Cooling and filtration of the mixture yielded 6.25 g of a white solid which was recrystallized from H_2O/95% EtOH to give 2.38 g (47%) of **105**, 90% deuterated by ^1H NMR; IR (KBr) 3410, 3100 (br), 2800 (br), 1635, 1600, 1510 cm^{-1}; ^1H NMR (D_2O, 80 MHz) δ

3.93 (m, 0.2 H, CH), 1.93 (m, 4H, CH_2CH), 1.63 (m, 2H, CH_2); MS (FAB) 193 (MH^+).

Decarboxylation of 105 in H_2O to [2,6-$^2H_2$]-L-Lysine.

This was carried out by Dr. M.M. Palcic. A typical reaction mixture consisted of 0.2 units of wheat germ or 1.4 units of B. sphaericus meso-diaminopimelate decarboxylase in 20 mM potassium phosphate buffer, pH 7.0 (50 mL), containing 0.01% 2-mercaptoethanol, 0.1 mM pyridoxal phosphate and [2,6-$^2H_2$]-2,6-diaminoheptanedioic acid (105) (500 mg). After 140 h at room temperature (26°C), lysine and 2,6-diaminoheptanedioic acid were removed by ultrafiltration and separated by ion-exchange chromatography as described above.

[2,6-$^2H_2$] (2S)-Methyl 2,6-bis([(1S,4S)-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carbonyl]amino)hexanoate (106)

The procedure described for the preparation of the monodeuterated derivative was followed to give 16-19% yields of the dideuterated derivative 106, from [2,6-$^2H_2$] L-lysine obtained from incubation of 105 with both Bacillus sphaericus and wheat germ decarboxylases. The samples from both sources had spectral and chromatographic properties identical with each other; IR ($CHCl_3$ cast) 3370, 1790, 1745, 1670, 1530 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) identical to that of 102, except for the loss of the multiplets at δ 4.73 (CH) and δ 3.26 (H_S); ^{13}C NMR (C_6D_6, 100.6 MHz) identical to that

of 102, except for the loss of the signals at δ 52.1 and δ 38.8 and the introduction of triplets at δ 52.2 and δ 38.7; exact mass 522.2909 (522.2910 calc. for $C_{27}H_{38}D_2N_2O_8$).

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